



T.C.
NİĞDE ÖMER HALISDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

ASSOCIATION MAPPING OF SOME AGRONOMIC AND MORPHOLOGICAL
TRAITS IN POTATO (*Solanum tuberosum* L.)

MUHAMMAD ABU BAKAR ZIA

July 2018

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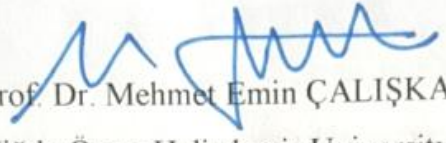
MUHAMMAD ABU BAKAR ZIA

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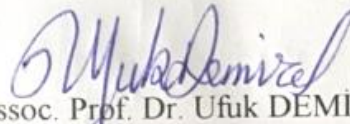
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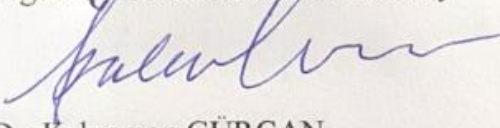
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The study titled “**Association Mapping of Some Agronomic and Morphological Traits in Potato**” presented by **Muhammad Abu Bakar ZIA** with the help of supervisor **Prof. Dr. Mehmet Emin ÇALIŞKAN** has been accepted as Doctoral thesis by the jury at the **Department of Agricultural Genetic Engineering** of Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.

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THESIS CERTIFICATION

I certify that the thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.



MUHAMMAD ABU BAKAR ZIA

ÖZET

PATATESTE (*Solanum tuberosum* L.) BAZI AGRONOMİK VE MORFOLOJİK ÖZELLİKLERİN İLİŞKİ HARITALAMASI

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Tarımsal Genetik Mühendisliği Anabilim Dalı

Danışman :Prof. Dr. Mehmet Emin ÇALIŞKAN

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Yeni nesil dizileme (NGS) tekniklerindeki son gelişmeler, DNA dizilemesini hem daha hassas hale getirmiş hem de maliyetini aşağı çekmiştir. Farklı ıslah programlarından temin edilen 237 farklı patates genotipi kullanılarak yapılan bu çalışmada, esas olarak patateste 20 önemli agronomik ve morfolojik özellik açısından bir ilişki haritası oluşturulması amaçlanmıştır. Ayrıca patates ıslah programlarında kullanılabilecek, her bir özelliğe ilişkili özgün SNP markörlerinin bulunması hedeflenmiştir. İlave olarak tetraploid patateste ilişki haritalaması çalışmaları için farklı istatistiksel modellerin karşılaştırılması yapılmıştır. Fenotipik verilerin elde edildiği tarla denemeleri, 2016 ve 2017 yıllarında beş kontrol çeşidi kullanılarak augmented deneme desenine göre göre yapılmıştır. Genel lineer model, popülasyon yapısı ve ilişki analizi içeren karışık lineer model kullanılarak, 16 farklı SNP markörünün genotiplerimiz ile ilişkili olduğu tespit edilmiştir. Bazı özellikler için ise hiçbir ilişki saptanmamıştır. Bu çalışma, özgün ve daha önce bilinmeyen gen ve markörlerin belirlenmesinde, polimorfizmlerin genom boyu tespiti ile bilgi temelli bir ilişki haritalamasının birleştirilmesi yaklaşımının önemini ortaya koymuştur. Bu çalışmada bulunan markörler, doğrulama çalışmalarından sonra patates ıslah çalışmalarında aday markörler olarak kullanılabilirler.

Anahtar Sözcükler: Yeni nesil dizileme, ilişkilendirme haritası, SNP, patates

SUMMARY

ASSOCIATION MAPPING OF SOME AGRONOMIC AND MORPHOLOGICAL TRAITS IN POTATO (*Solanum tuberosum* L.)

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Graduate School of Natural and Applied Sciences
Department of Agricultural Genetic Engineering

Supervisor :Prof. Dr. Mehmet Emin ÇALIŞKAN

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Recent progress in next generation sequencing (NGS) techniques has made DNA sequencing as high throughput and very cost effective. This study is mainly aimed to perform an association mapping in potato for 20 important agronomic and morphological traits using 237 diverse potato genotypes from different breeding programs. It was aimed to explore novel Single Nucleotide Polymorphism (SNP) markers which could be used as selection marker in potato breeding programs related with each trait in potato. It was also compared the effectiveness of different statistical models for association mapping studies in tetraploid potato. Field experiments for phenotypic data were conducted in 2016 and 2017 in augmented design using five check cultivars. Using a general linear model, mixed linear model including population structure and kinship for association analysis, 16 different SNP markers were found to be associated to the phenotypic trait. Some of the traits also remained unassociated. This study shows the value of combining a knowledge-based association mapping approach with genome-wide discovery of polymorphisms as a tool for the detection of novel and non-obvious candidate genes and markers. The markers found in this study can be used as candidate markers after validation for potato breeding studies in the future.

Keywords: Next generation sequencing, association mapping, SNP, Phenotyping, potato

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TABLE OF CONTENTS

ÖZET	iv
SUMMARY	v
ACKNOWLEDGMENTS	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF SYMBOLS AND ABBREVIATIONS	xvi
CHAPTER I INTRODUCTION.....	1
CHAPTER II REVIEW OF LITERATURE	4
2.1 History of Potato Breeding	4
2.2 Marker Technology.....	6
2.2.1 Amplified Fragment Length Polymorphism	7
2.2.2 Microsatellites or Simple Sequence Repeats	8
2.3 Quantitative Trait Loci Mapping	9
2.4 Potato QTL Analysis	10
2.5 SolCAP SNP Array.....	11
2.6 Association Mapping	12
2.7 Association Studies for Potato	15
2.8 Analysis of Population Structure to Prevent False-positives.....	17
2.9 Applications of Association Mapping	17
2.10 Advantages of Association Mapping over Linkage Mapping	19
2.11 Approach to Anatomize the Quantitative Traits	20
2.11.1 Analysis using bulk segregants	20
2.11.2 Case control studies	21
CHAPTER III MATERIAL AND METHODS.....	23
3.1 Plant Traits	26
3.1.1 Growth habit	26
3.1.2 Branching pattern.....	26

3.1.3 Plant height	26
3.1.4 Stem thickness	26
3.1.5 Leaves shape	27
3.1.6 Number of leaflets	27
3.1.7 Maturity	27
3.2 Flower Traits	28
3.2.1 Flower color	28
3.2.2 Pistil length	28
3.2.3 Stamen length	28
3.2.4 Pistil length above stamens	28
3.3 Tuber Traits.....	29
3.3.1 Tuber skin color	29
3.3.2 Number of tubers per plant	29
3.3.3 Tuber shape.....	30
3.3.4 Tuber size.....	30
3.3.5 Tuber flesh color	30
3.3.6 Tuber skin texture	30
3.3.7 Tuber yield.....	30
3.4 Tuber Quality Traits.....	31
3.4.1 Specific gravity	31
3.4.2 Dry matter content	31
3.5 DNA Extraction and Genotyping	31
3.6 Population Structure and Association Mapping	33
3.6.1 General linear model.....	34
3.6.2 Mixed linear model.....	34
CHAPTER IV RESULTS AND DISCUSSION	35
4.1 Cluster Analysis	35
4.2 Dendrogram	36
4.3 Correlation Analysis	37
4.4 Linkage Disequilibrium	39
4.5 Association Mapping	43
4.5.1 Plant traits	43
4.5.1.1 Growth habit.....	43
4.5.1.2 Branching pattern	48
4.5.1.3 Plant height.....	52

4.5.1.4 Stem thickness.....	57
4.5.1.5 Leaves shape	61
4.5.1.6 Number of leaflets per plant.....	64
4.5.1.7 Maturity	68
4.5.2 Flower Traits.....	74
4.5.2.1 Flower color.....	74
4.6.2.2 Stamen length	77
4.6.2.3 Pistil length	81
4.6.2.4 Pistil length above stamen	84
4.5.3 Tuber Traits	88
4.5.3.1 Tuber skin color	88
4.5.3.2 Number of tubers per plant.....	92
4.5.3.3 Tuber shape	98
4.5.3.4 Tuber size	101
4.5.3.5 Tube flesh color.....	104
4.5.3.6 Tuber skin texture.....	107
4.5.3.7 Tuber yield	110
4.5.4 Tuber Quality Traits.....	114
4.5.4.1 Specific gravity	114
4.5.4.2 Dry matter content.....	119
CHAPTER V CONCLUSION.....	125
REFERENCES	128
APPENDIX.....	159
CURRICULUM VITEA.....	187

LIST OF TABLES

Table 3.1. List of genotypes studied	23
Table 4.1. List of genotypes included in separate cluster of cluster analysis	36
Table 4.2. Linkage Disequilibrium in the population used.....	42
Table 4.3. SNP found in 2016 data set from GLM for growth habit.....	44
Table 4.4. SNP found in 2017 data set from for growth habit GLM.....	44
Table 4.5. SNP found in 2016 data set from GLM for growth habit.....	45
Table 4.6. SNP found in 2016 data set from MLM for growth habit	47
Table 4.7. SNP found in 2016 data set from GLM for branching pattern	49
Table 4.8. SNP found in 2016 data set from GLM for plant height	55
Table 4.9. SNP found in BLUE data set from GLM for stem thickness	59
Table 4.10. SNP found in 2016 data set from GLM for maturity.....	71
Table 4.11. SNP found in 2017 data set from GLM for maturity.....	71
Table 4.12. SNP found in BLUE data set from GLM for maturity	71
Table 4.13. SNP found in 2016 data set from MLM for maturity	72
Table 4.14. SNP found in 2016 data set from GLM for flower color	74
Table 4.15. SNP found in 2016 data set from GLM for stamen length.....	78
Table 4.16. SNP found in 2016 data set from GLM for tuber skin color	89
Table 4.17. SNP found in 2017 data set from GLM for tuber skin color	89
Table 4.18. SNP found in BLUE data set from GLM for tuber skin color.....	89
Table 4.19. SNP found in 2016 data set from MLM for tuber skin color	91
Table 4.20. SNP found in 2016 data set from GLM for number of tubers per plant.....	94
Table 4.21. SNP found in 2017 data set from GLM for number of tubers per plant.....	94
Table 4.22. SNP found in BLUE data set from GLM for number of tubers per plant.....	94
Table 4.23. SNP found in 2016 data set from MLM for number of tubers per plant	96
Table 4.24. SNP found in BLUE data set from GLM for tuber size	102
Table 4.25. SNP found in BLUE data set from MLM for tuber size.....	104
Table 4.26. SNP found in 2016 data set from GLM for tuber yield.....	110
Table 4.27. SNP found in BLUE data set from GLM for tuber yield	111

Table 4.28. SNP found in 2016 data set from GLM for specific gravity	115
Table 4.29. SNP found in 2017 data set from GLM for specific gravity	115
Table 4.30. SNP found in 2016 data set from MLM for specific gravity.....	116
Table 4.31. SNP found in 2016 data set from MLM for specific gravity.....	117
Table 4.32. SNP found in 2016 data set from GLM for dry matter content.....	120
Table 4.33. SNP found in BLUE data set from GLM for dry matter content	120
Table 4.34. SNP found in 2016 and 2017 data set from MLM for dry matter content.....	122
Table 4.35. SNP found in BLUE data set from MLM for dry matter content.....	123



LIST OF FIGURES

Figure 2.1. The scheme of association mapping.....	19
Figure 3.1. Crop for the growing season of 2016	25
Figure 3.2. Crop for the growing season of 2017	26
Figure 3.3. Different leaf shapes in potato crop	27
Figure 3.4. Different flower colors in potato crop from different genotype lines	28
Figure 3.5. Digital Compass to the measure the traits related to stamen and pistil length in potato flower	29
Figure 3.6. Martin Lishmans's digital potato Hydrometer used to measure specific gravity and dry matter content of potato crop.....	31
Figure 4.1. Cluster analysis of the genotypes	35
Figure 4.2. Dendrogram of the phenotypes prepared using genotype data	37
Figure 4.3. Correlation Analysis of the traits.....	38
Figure 4.4. Triangle plot for pairwise LD between SNP markers	40
Figure 4.5. LD decay plot portrayed from LD values	41
Figure 4.6. Q-Q plot for growth habit of potato for the year 2016, 2017 and BLUE.....	43
Figure 4.7. Manhattan plots from generalized linear model for growth habit of potato plants	46
Figure 4.8. Manhattan plot through mixed linear model for the year 2016, 2017 and BLUE.....	47
Figure 4.9. Q-Q plot for the trait branching pattern of potato crop	49
Figure 4.10. Manhattan plots from generalized linear model for branching pattern	50
Figure 4.11. Manhattan plots from mixed linear model for the trait branching pattern	51
Figure 4.12. Bar plots for the year 2016, 2017 and BLUE for plant height	52
Figure 4.13. Q-Q plot for plant height for potato crop for the year 2016, 2017 and BLUE	53
Figure 4.14. Manhattan plots as per GLM for the trait plant height.....	54
Figure 4.15. Manhattan plots as per MLM for plant height trait of potato.....	56
Figure 4.16. Bar plots for stem thickness traits for the year 2016, 2017 and BLUE.....	57
Figure 4.17. Q-Q plots for stem thickness for the year 2016, 2017 and BLUE	58

Figure 4.18. Manhattan plots for stem thickness using GLM	59
Figure 4.19. Manhattan plots for the trait stem thickness using MLM	60
Figure 4.20. Q-Q plot for leaves shapes trait of potato	61
Figure 4.21. Manhattan plots for leaves shapes using GLM	62
Figure 4.22. Manhattan plots for leaves shapes using MLM.....	63
Figure 4.23. Bar plots for number of leaflets per plant.....	64
Figure 4.24. Q-Q plots for number of leaflets per plant for the year 2016, 2017 and BLUE	65
Figure 4.25. Manhattan plots obtained from GLM for the trait number of leaflets per plant	66
Figure 4.26. Manhattan plots obtained from GLM for the trait number of leaflets per plant	67
Figure 4.27. Bar plots for maturity of the potato plants	68
Figure 4.28. Q-Q plots for maturity of the plants for the year 2016, 2017 and BLUE	69
Figure 4.29. Manhattan plots obtained from GLM for maturity	70
Figure 4.30. Manhattan plots obtained from MLM for maturity of the potato plants	73
Figure 4.31. Q-Q plots for the year 2016, 2017 and BLUE for the trait of flower color	74
Figure 4.32. Manhattan plots using Generalized Linear Model for Flower color	75
Figure 4.33. Manhattan plots using MLM for Flower color trait in potato	76
Figure 4.34. Bar plots for the year 2016, 2017 and BLUE for the trait Stamen length	77
Figure 4.35. Q-Q plots for the trait anther length in potato plants	78
Figure 4.36. Manhattan plots using Generalized Linear Model for Stamen length trait in potato	79
Figure 4.37. Manhattan plots using MLM for Stamen length trait in potato.....	80
Figure 4.38. Bar plots for the trait Pistil length for the growing season of 2016, 2017 and BLUE	81
Figure 4.39. Q-Q plots for the trait Pistil length for 2016, 2017 and BLUE	81
Figure 4.40. Manhattan plots using Generalized Linear Model for Pistil length trait in potato	82
Figure 4.41. Manhattan plots using Mixed Linear Model for Pistil length trait in potato	83
Figure 4.42. Bar plots for the trait Pistil length above stamen for potato plants	84

Figure 4.43. Q-Q plots for the trait Pistil length above stamen for potato plants.....	85
Figure 4.44. Manhattan plots using Generalized Linear Model for Pistil length above stamen trait in potato	86
Figure 4.45. Manhattan plots using Mixed Linear Model for Pistil length above stamen trait in potato.....	87
Figure 4.46. Q-Q plots for 2016, 2017 and BLUE for tuber skin color in potato	88
Figure 4.47. Manhattan plots using Generalized Linear Model for tuber skin color trait in potato	90
Figure 4.48. Manhattan plots using Mixed Linear Model for tuber skin color in potato	91
Figure 4.49. Bar plots for number of tubers per plant for the year 2016, 2017 and BLUE	93
Figure 4.50. Q-Q plots for number of tuber per plant traits in potato for the year 2016, 2017 and BLUE.....	93
Figure 4.51. Manhattan plots using Generalized Linear Model for number of tuber per plant trait in potato for the year 2016, 2017 and BLUE	95
Figure 4.52. Manhattan plots using MLM for number of tuber per plant trait in potato	97
Figure 4.53. Q-Q plots for tuber shape in potato for the year 2016, 2017 and BLUE	98
Figure 4.54. Manhattan plots using Generalized Linear Model for tuber shape in potato	99
Figure 4.55. Manhattan plots using Mixed Linear Model for tuber shape trait in potato	100
Figure 4.56. Q-Q plots for the trait of tuber size for the year 2016, 2017 and BLUE.....	101
Figure 4.57. Manhattan plots using Generalized Linear Model for tuber size in potato	102
Figure 4.58. Manhattan plots using Mixed Linear Model for tuber size trait in potato	103
Figure 4.59. Q-Q plots for tuber flesh color of potato for the year 2016, 2017 and BLUE	104
Figure 4.60. Manhattan plots using Generalized Linear Model for tuber flesh color trait in potato	105
Figure 4.61. Manhattan plots using MLM for tuber flesh color in potato	106

Figure 4.62. Q-Q plot for tuber skin texture of potato tuber for the year 2016, 2017 and BLUE	107
Figure 4.63. Manhattan plots using Generalized Linear Model for tuber skin texture trait in potato.....	108
Figure 4.64. Manhattan plots using Mixed Linear Model for tuber skin texture trait in potato	109
Figure 4.65. Q-Q plots for tuber yield of potato crop for the year 2016, 2017 and BLUE	110
Figure 4.66. Manhattan plots using Generalized Linear Model for tuber yield in potato.....	112
Figure 4.67. Manhattan plots using Mixed Linear Model for tuber yield trait in potato.....	113
Figure 4.68. Bar plot for the trait specific gravity of potato crop for the year 2016, 2017 and BLUE	114
Figure 4.69. Q-Q plot for specific gravity of potato crop for the year 2016, 2017 and BLUE	115
Figure 4.70. Manhattan plots using GLM for specific gravity in potato.....	116
Figure 4.71. Manhattan plots using Mixed Linear Model for specific gravity in potato	118
Figure 4.72. Bar plots for dry matter content of potato crop for the year 2016, 2017 and BLUE	119
Figure 4.73. Q-Q plot for dry matter content of potato crop for the year 2016, 2017 and BLUE.....	119
Figure 4.74. Manhattan plots using GLM for dry matter content of potato	121
Figure 4.75. Manhattan plots using MLM for dry matter content of potato.....	122

SYMBOLS AND ABBREVIATIONS

Symbols

Descriptions

%	Percent sign
°C	Degree Centigrade
bp	Base Pair

Abbreviations

Description

FAOSTAT	Food and Agriculture Organization Statistical Databases (United Nations)
AM	Association Mapping
QTL	Quantitative Trait Loci
LD	Linkage Disequilibrium
SolCAP	Solanaceae Cooperative Agricultural Project
GLM	Generalized Linear Model
MLM	Mixed Linear Model
TASSEL	Trait Analysis by aSSociation, Evolution and Linkage
SNP	Single Nucleotide Polymorphism
AFLP	Amplified Fragment Length Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SSRs	Simple Sequence Repeats
cM	Centi Morgan
DNA	Deoxyribonucleic acid

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) as being the significant crop universally maintains forth position among food producing crops following maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivum* L.) (FAOSTAT, 2018). Potato is a tetraploid and extremely heterozygous crop. It also has the foremost importance as food, feed and industrial raw materials. In Turkey, potato in gross national product (GNP) occupies 3% of the total GNP, while in EU-27 countries it is about 3.1%, hence it backs meaningfully in the economy of Turkey (Çalışkan et al., 2010). In 2017, potato was grown on 143,000 ha with outcome of 4.800 million tons in Turkey (TÜİK, 2017). Around 60% of Turkey's potato production is from Central Anatolia including Niğde having vast field of cultivation throughout the country and a vital part in agriculture sector of Turkey. Likewise, in Pakistan, it is cultivated over 170,000 ha with an annual outcome of 3.8 m tons -(Economic Survey of Pakistan, 2014-15). For security of food and social sustainability in future, as potato is a highly nutritious crop, viable potato production techniques are vital. Potato is thought to be the most important crop for the reduction of hunger as well as poverty throughout the world due to the high nutritional potential of the crop. Potato has a high amount of proteins, carbohydrates (starch), vitamins (C, B1, B3, B6, folate, pantothenic acid and K) and also minerals (K, P, Mg, Mn, Fe, Cu) (Çalışkan et al., 2010).

Start of potato cultivation was mainly through domestication of single *Solanum tuberosum* in the southern part of Peru (Spooner et al., 2005). Spaniards carried potato to Spain while they were getting back to their homeland from South America about 1570s. Initially, the potato was grown as ornamental plant in gardens due to its beautiful flowers. The cultivation of potato in Europe was indicated for food in letter 1577 (Oliemans, 1988). Earlier, potato was only the bread for poor people (Oliemans, 1988). Currently, it has gain the value of an important commodity in diets all over the world.

Cultivated European potatoes *Solanum tuberosum* (ssp. *tuberosum*) have four alleles per locus that's why they are termed as tetraploid ($2n=4x=48$). Moreover, the homologous chromosomes pair during the process of meiosis, due to that they are called as autotetraploid (Milbourne et al., 2007). Besides from the tuber bearing cultivated potato

species, there are some non-tuberosum species which range from diploid to hexaploid in their ploidy level (Van den Berg and Jacobs, 2007). Potato is highly heterozygous and it makes potato very vulnerable to inbreeding, shaping them hard to get or go for homozygous lines. Heterozygous nature of potatoes especially in the cultivars that are commercially being dealt is maintained through repeated propagation of clones (The Potato Genome Sequencing Consortium, 2011; Milbourne et al., 2007).

Variations in natural DNA sequences of the individuals caused the formation of different molecular marker techniques (Gebhardt, 2005). Over the last few decades numerous markers have been formulated for the visualization of these variations in the genome of the crop. In restriction enzyme recognition sites, first molecular markers were established that were based on polymorphisms and were named as Restriction Fragment Length Polymorphism (RFLP) (Botstein et al., 1980). Marker system that is based on PCR were followed by RFLP's, for example Random Amplification of Polymorphic DNA (RAPD), microsatellite (SSR) (Williams et al., 1990; Welsh and McClelland, 1990), Amplified Fragment Length Polymorphisms (AFLP) markers (Vos et al., 1995), SSCP (single-strand conformation polymorphism) markers and cleaved amplified polymorphic sequences (CAPS) markers that are based on PCR. Sequencing techniques, like Sanger amplicon sequencing, later permitted the detection of polymorphism at single nucleotide (SNPs) and that is why the allelic dosage assessment at single nucleotide polymorphism loci for specific genotypes became pertinent. Potato contains five (5) different type of possible mixture of SNPs at bi-allelic level: two of them are homozygous in nature (i.e. nulliplex, quadruplex) and three combinations are heterozygous (i.e. simplex, duplex and triplex).

Recently, SolCAP Potato Array has been established with the immense increase in the high throughput genotyping assays (Hamilton et al., 2011; Felcher et al., 2012). With the establishment of SolCAP Array, with the use of SolCAP SNP array of 8K, 12K and 69K at considerably low price potato using these high-density genotyping techniques, genotyping can be performed. Large number of SNPs per genotype can easily be analyzed with the help of SolCAP Potato Custom-made arrays (Hamilton et al., 2011; Felcher et al., 2012). With total number of 8, 12 and 69K SNP markers, Concurrent genotyping of any individual can be performed with the help of SolCAP Potato Array. Sequences of five North American potato varieties (Premier Russet, Kennebec, Atlantic, Shepody and Snowden) one of the European variety (Bintje) were used in the

development of this SNP array. Next generation sequence of transcriptome was used for the development of sequence data for the cultivars Atlantic, Premier Russet and Snowden. Moreover, other sequences were also taken from different databases at public level for the varieties like Bintje, Shepody, Kennebec (Hamilton et al., 2011). In an array, 36% of the region was taken from marker loci of candidate gene of interest of all the markers, 6% from previously mapped markers and remaining 57% of the markers were elected for a higher analysis of the potato genome in the coding regions (Felcher et al., 2012). Until now, fewer reports have been presented enforcing the SNP SolCAP Potato Array. Felcher et al., (2012) described the sketch and the assimilation of diploid linkage maps with genome sequence of potato. SolCAP Potato Array helped in a genotyping of a bi-parental tetraploid mapping population (Hackett et al., 2013). As a pilot study it was first applied on European potato germplasm Stich et al., (2013), where population structure (Q) and linkage disequilibrium (LD) of 36 different tetraploid cultivars and eight diploid potato clones were tested.

Augmentation of huge segregating population of more than 200 lines is required for linkage mapping in potato as potato being the autotetraploid in nature (Hackett et al., 2001) as well as huge sum of markers are also obligatory as compared to the diploid ones. Alternatively, association mapping is quite attractive; it is quick as accumulations of lines are not a big deal. We can use already available genotypes rather to spend our time in creating a specific mapping population. Association mapping also scrutinize all the variations on allelic level from numerous different meiotic events taking place in breeding programme instead of couple of parents in a mapping population and only because of this one can have a higher resolution as compared to experiments in conventional linkage mapping (Rafalski, 2002; Buckler and Thornsberry, 2002). Many studies have been conducted for association mapping in potato, for example, Simko et al., (2004), Li et al., (2005), Malosetti et al., (2007) and Gebhardt et al., (2004). This study was aimed to explore novel SNP markers related with the traits, effectiveness of different statistical methods and to identify significant markers which could be used for future potato breeding programs.

CHAPTER II

LITERATURE REVIEW

2.1 History of Potato Breeding

Potato (*Solanum tuberosum* L.) is the 4th important food crop around the world in case of production following maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivum* L.) (FAOSTAT, 2018). Potatoes were invented from Peruvian Andes from a single domestication practice (Spooner et al., 2005). Story of its expansion started with the Spanish conquistadores, as they took potato to the Europe as a “botanical” innovation from the “New World”. Salaman, (1949) and Glendinning, (1983) believed that presumably their beginning started somewhere in the region of north Andean (Colombia/Venezuela) and that also that there are two distinct introductions of potatoes in late 16th century. The introduction of potatoes to the new region came with an uneven shape with defacing outgrowths along with deep-eyes (Salaman, 1926), which contradicted with the up-to-date cultivars that have shallow-eyed and having shallow eyes. Potato was thought to be a garden crop by the botanists; in monasteries originally and even up to 18th century they were ignorant of increased cultivation from potato as a food crop (Oliemans, 1988). Potato was started to be considered as one of the major European food crops by the start of early 19th century. This development in potato also encouraged the disease like virus, nematode, and the overwhelming disease of late blight that was the cause of great Irish Famine in 1847, elicited the exercise for potato breeding (Glendinning, 1983).

Earlier the start of 1850, selection was used to be practiced among seedlings. Mostly the plantlets were being grown from the barries and the seeds which are the result of the crosses made between the two parents. The pivotal traits that were addressed the most during that duration were day-length adapted for crop yield, size of tuber and shape of the tuber. With the passage of time and by 19th century well documented germplasm introduction to widen the gene pool from Latin America that was destroyed because of the increase in late blight disease. Rough Purple Chili is an example that represents the ancestors of potato both in Europe and USA that have the remarkable list of varieties in their pedigree (van Berloo et al., 2007; Plaisted and Hoopes, 1989; Glendinning, 1983). Ortiz, 1998 explained very comprehensively that Rough Purple Chili is from the gene pool of USA. Garnet Chili was selected from seedlings cultivars that were open-

pollinated. Then Garnet Chili found to be Early Rose parent, which stands along the descendants of Russet Burbank which is among the commonly cultivated cultivars of United States of America. A scan of database of pedigree of potato (http://www.plantbreeding.wur.nl/potato_pedigree), deep up to 25 generations, means that 60% of the pedigrees of Early Rose are present among 7,074 accessions. Therefore, we can consider Rough Purple Chili among the few but powerful originators of potatoes that are being cultivated now-a-days. After 1850, breeders started the crossing between the potato cultivars most. To improve the pathogens resistance in potato with the help of repeated back crossings in wild species germplasm took place in 20th century. Though present-day potato cultivars generally belong to wild relatives and archaic cultivars forebearers, just because of the selection of about 80% of the genes that they had are the outcome of a very few number of cultivars that were being cultivated early in 20th century (Glendinning, 1983).

Because of their narrow genetic base, the modern commercial potato cultivars are highly interconnected (Love, 1999), but also due to the reason that less amount of sexual generations since the onset of present-day breeding program. Moreover, recombination rate is restricted to not less than one cross-over per chromosome hardly, which results in sparing 50% of all the chromatids present that had no recombination (van Os et al., 2006). Thus, it's normal that linkage disequilibrium is more stretched in the potato genome as compared to the other outbreeding crops like maize. This suggests in a very comprehensive way that fine mapping perhaps will remain ideal for potato where we can increase the mapping resolution by using a bunch of more genetically erratic cultivars when compared with the mapping population of full-sib category that are normally being implemented in Quantitative Trait Locus (QTL) studies even if the methodology association mapping is used.

There are a lot of suggestions to improve the potato breeding (Bradshaw and Mackay, 1994), but all of them remained unsuccessful to that can change the breeding practices that are commercially being practiced up till now, typically the tetrasomic inheritance is the cause behind this reason. The reproduction rate of vegetatively propagated potato through seed tubers and with clonal selection of many years is in shrill distinction as compared to the other crops. When both hemispheres are used in the crops that are being grown at greenhouse, for example, tomato or cucumber, in a year up to four selection cycles can easily be achieved. Use of parthenogenetic development of

unfertilized eggs to broaden the genetic base and to introduce unique genetic diversity from wild relatives breeding at diploid level by in amalgamation with complete set of gametes has been suggested for a feasible resolution in potato (Hougas and Peloquin, 1958). By the introduction modern techniques like protoplast fusion and embryo rescue it has become possible to get the desirable results even through obscure associated wild relatives that are not suitable to present-day tetraploid potato (Bradshaw and Mackay, 1994; Ortiz, 1998). It's very hard to get homozygous potato genotype mainly due to the immense inbreeding depression, creating vigor and fertility loss and many tries for making potato a true homozygous by consecutive selfing remained unsuccessful (Douches et al., 1996). Mutation breeding is a method that is effectively implemented in ornamentals being propagated asexually is correspondingly dubious to be a forthright substitute to common practices in potato breeding as mutations will be disguised with the action of other alleles as being tetraomic inheritance, though there are some success cases that have been reported for yield, late blight resistance as well as for starch properties (Kowalski and Cassells, 1999; Muth et al., 2008; Jacobsen et al., 1989).

With the beginning of linkage maps and molecular markers era, Marker Assisted Selection (MAS) arose like a beneficial engine both for plants as well as for the animals in breeding programs. Unambiguously, marker assisted selection is beneficial for potato crop specially, no matter it is applied for positive selection as well as for negative selection – say for example to accelerate the rehabilitation of *tuberosum* linked background when introducing the characteristics from wild relatives, to pursue a specific number of characteristics at the same time among selection in early production or for the screening of whole breeding programme for interlinked traits to support the parental choice. Barone, (2004) presented the summary of the results of marker assisted selection within the potato.

2.2 Marker Technology

Visualizing DNA sequence variations at specific gene locus, molecular marker techniques are being used. These molecular marker means have been formulated in the past 20 to 30 years at the time when sequencing of DNA was almost impossible specially to produce an abundant data. The pioneer markers used for restriction enzymes in the recognition sites were the differences in DNA sequence.

Polymorphic pattern that were interpreted for representing RFLP marker locus was the results of the changes in length between the restriction fragments. With the introduction of PCR machine in the field of molecular genetics, another series of methods were produced utilizing variations in lengths in the PCR product among the primer annealing site and/or restriction site polymorphism. Multi locus marker techniques replaced single locus marker techniques. And due to which Amplified Fragment Length Polymorphism (AFLP) has acquired a wide adoption in molecular genetic studies.

2.2.1 Amplified Fragment Length Polymorphism (AFLP)

AFLP was first elaborated by Vos et al., 1995, AFLP markers are deep rooted and commonly used marker technique that is being used in mapping, breeding applications as well as in the studies related to genetic diversity in the organisms of vast range irrespective of ploidy level as well as plants and animals (Meudt and Clarke, 2007). The technique for AFLP markers is based on the digestion of genomic DNA which results in selective PCR amplification of restriction fragments. Archetypally, almost 100 fragments are amplified per reaction per each combination of primer. Polymorphism extent is the result of occasional additions and deletions between the sequences of specific primers (Vos et al., 1995). Amplified Fragment Length Polymorphism (AFLP) is high reproducible, have the coverage over the whole genome, cost-effectiveness is due to an increased ration of multiplex. It also does not need the earlier instructions regarding the sequence of DNA (Meudt and Clarke, 2007; Breyne et al., 1997; Powell et al., 1996; Karp et al., 1996). Uncommon genome size can also be dealt easily with this technique (Han et al., 1999).

Nevertheless, when the polyploid species like potato are considered, Amplified Fragment Length Polymorphism (AFLP) has the drawback that it cannot take much information to the researcher about the marker data that is dominantly scored (i.e. either presence/absence) when correlated to the techniques of maker that is co-dominant in nature. Although this feature of AFLP, some researches are there that used band intensities to score AFLP in a semi co-dominant manner. Among the pioneer effort that were accomplished by Castiglioni et al., (1999) using maize of diploid nature utilizing the software of image analysis to measure the intensities of bands and changed these values into band zygosity. Co-dominant AFLP marker data can be taken by applying blended models to a system that is bases on gel or intensities of fluorescence band in

capillary systems on optical band densities, Piepho and Koch, (2000) used as model experiment in diploid sugar beet as well as it was conducted in tomato by the personal communication of Gort et al., Still, conceding areas carry through allocation of the diploid heterozygous and diploid homozygous genotypes, for that reason a decent genotyping is a continuing concern in prevailing conditions (Jansen et al., 2001), mostly definite fragments proportion are trustworthy for scoring in a co-dominant way (Meudt and Clarke, 2007). No seeming deficit in precision at risk while efforts are being made to achieve measurable data from band intensities of AFLP. Lowest possible situation, both the quantitative and qualitative methods will function evenly, i.e. present/not being present method (Piepho, 2001). If scoring is done in a co-dominant way, AFLP motif would be strong to be accomplished on daily basis, so that effectiveness of expenditure will increase to even large scale and the genome of the polyploid species can easily be approachable, this definitely will provide a boost in a growth of significant stimulus for plant sciences (Meudt and Clarke, 2007).

2.2.2 Microsatellites or Simple Sequence Repeats (SSRs)

Simple Sequence Repeats (SSRs) or Microsatellites are the marker system along with PCR reaction was stated initially in the year 1989 by three different sovereign groups simultaneously (Tautz, 1989; Litt and Luty, 1989; Weber and May, 1989). Simple Sequence Repeats have loci of polymorphic nature that contain repeated parts of 1-6 base pairs length are existing in nuclear and organelles DNA and are encircled with uncommon sequences of DNA on which the particular DNA primers can be planned (Cregan and Quigley, 1997). Usual processes that cause the repeated number of variation is mainly due to the slippage of polymerase during the process of DNA replication or DNA repair (Tautz, 1989).

Akkaya et al., (1992) initially explained the microsatellite markers and described the usefulness of microsatellite markers in identification of the genotypes, mapping studies and population studies within the plants especially in soybean and it was implemented initially from Bell and Ecker, (1994) for *Arabidopsis thaliana* linkage mapping studies. After that these were started to be used in various fields, like association mapping, genome mapping, comparative mapping and tagging, functional genomics, QTL mapping, diversity related studies of the gene are some of them as reported by Varshney et al., (2005) in his review. Benefits of SSRs are placed in their reproducible capacity,

multi-allelism, co-dominance in nature, genome wide inclusion, multiplex possibility and diverse application (Varshney et al., 2005; Sharopova, 2008; Gupta and Varshney, 2000). SSRs also have some drawbacks: like having stammer bands and ineffectual alleles (Varshney et al., 2005). Over-or disparagement of real materiality between the species can be caused by the multi-allelic character of microsatellites (Provan et al., 1996a). Though, prevailing understanding challenges does not minimize the circumstances of dealing with microsatellite markers (McGregor et al., 2000). Recently it was proposed that there is even more important role as it was known before especially for SSR markers rather to be used only mapping in breeding: its affirmation that SSRs are participating in transcriptional and post-transcriptional gene regulation expression and can also be participating in regulation of recombination (Kashi and King, 2006; Kashi et al., 1997).

In potato, microsatellite marker was first formulated by Kawchuk et al., (1996) and Provan et al., (1996a, 1996b). After initial progress, a large set of microsatellites markers was formulated. Milbourne and colleagues (1997) formulated pairs of 112 SSR primer among them a small set of six potato clones contained 98 SSRs showed to be polymorphic. Ghislain et al., at International Potato Center (CIP) Peru conducted an experiment in which they tried 70 already known SSR loci on potato cultivar samples coming from nine different groups of taxonomy for delivery of SSRs among commonly grown species of potato and recommended small set of 18 high quality identification kit of potato genetic markers (Ghislain et al., 1999). In the year 2005, another 61 EST- arised SSRs were joined the already present lot of about 100 SSRs that were designed for potato crop (Feingold et al., 2005). Mapping of SSRs are conveniently obtained for the mapping population of diploid species, although the practice of these microsatellite or SSR markers in tetraploid populations is still not that easily understandable and to the point (Lander gott et al., 2006; Esselink et al., 2004).

2.3 Quantitative Trait Loci (QTL) Mapping

Linkage of the molecular markers that are segregating with data of phenotypic trait in the population of full sib permits finding of the loci that are participating in the traits that are quantitative in nature is called QTL mapping (Paterson et al., 1988). Intrinsically, analysis of QTL helps disclosing the linkage between a specific marker and quantitative trait. QTL mapping might be accomplished with the help of mapping

populations like Recombinant Inbred Line (RILs), F2, Doubled Haploid as well as by Back Cross populations (Kearsey, 1998; Kearsey and Farquhar, 1998; Collard et al., 2005). There is much work that has been done on the strategies of QTL mapping and was bestowed through Borevitz and Chory, (2004), Mackay, (2001), Kearsey and Farquhar, (1998), and Tanksley, (1993).

2.4 Potato QTL Analysis

Diploid mapping populations have been utilized in potato to perform for QTL mapping studies as being statistically better. Loci responsible for late blight resistance (Visker et al., 2003; Costanzo et al., 2005; Bradshaw et al., 2006; Collins et al., 1999; Leonards-Schippers et al., 1994; Park et al., 2005; Trognitz et al., 2002), nematodes responsible for potato cyst disease (Roupe van der Voort et al., 1998, 2000; Kreike et al., 1993; Bryan et al., 2002), *Verticillium albo-atrum* (Simko et al., 2004b), insect resistance that is arbitrated with trichome (Bonierbale et al., 1994) as well as thrips resistance (Galvez et al., 2005) have successfully been mapped for mapping population of diploid species. Similarly, QTL that deal with the quality and agronomic traits of potato as, dormancy of the crop and specific gravity (Freyre et al., 1994; Freyre and Douches, 1994; Ewing et al., 2004), tuberization for the specific crop cultivar (Fernandez-Del-Carmen et al., 2007), potato leaf aging (Malosetti et al., 2006), process of chlorosis (Simko et al., 2008), shape of tuber, regularity in shape of tuber, potato eye depth and tuber flesh color (van Eck et al., 1994a,b ; Sliwka et al., 2008), cold sweetening (Menendez et al., 2002), discoloration caused by enzymatic activities (Werij et al., 2007), chip color (Douches and Freyre, 1994), glycol-alkaloid content (Yencho et al., 1998; Sørensen et al., 2008), starch and yield content of the crop (Schäfer-Pregl et al., 1998) have successfully been discovered in diploid population, commonly interspecific species and those having a genetic back ground.

If it is difficult for a molecular marker map to be, just a few QTL researches are conducted using mapping population tetraploid in nature. Perpetually those works were established on the mapping population tetraploid potatoes i.e. 12601ab1 x Stirling, which was grown at the Institute of Scottish Crop Research. So far QTL's for the traits like late blight, yield, resistance against potato cyst nematode, agronomic as well as the traits related to quality have been identified (Bradshaw et al., 2008; 2004; 1998). Additional reports capitalizing the population that was tetraploid for mapping studies

stated few groups with limited linkage with the traits that are based either on a single gene or on major QTL. As an illustration, considerable QTLs have been situated for the resistance against the disease of corky ring spot (Khu et al., 2008), gene Rx for resistance against X virus in potato (Bendahmane et al., 1997), as well as R2 ramified in the resistance against late blight disease (Li et al., 1998).

2.5 SolCAP SNP Array

Recently, SolCAP SNP array has been developed (Felcher et al., 2012; Hamilton *et al.* 2011), Which is now a days being widely used in potato research (Hirsch et al., 2013; Manrique-Carpintero et al., 2014; Prashar et al., 2014). SNPs like this, requires the study about discovery, which is enabled with the help of next-generation sequencing. Various methods for SNP discovery are being practiced like (1) re-sequencing of whole genome (Yamamoto et al., 2010), (2) sequencing of the transcriptomes (Barbazuk et al., 2007; Hamilton et al., 2011, 2012; Trick et al., 2009) and (3) restriction enzymes-based sequencing (Baird et al., 2008). With the establishment of SolCAP Array, we can perform genotyping of potato germplasm with 8K, 12K and now 69K SNPs at considerably less price using these high-density genotyping techniques. Large number of SNPs per genotype can easily be analyzed with the help of SolCAP Potato Custom-made arrays (Hamilton et al., 2011). In the development of SolCAP SNP array, sequence of five North American potato cultivars (Premier Russet, Kennebec, Atlantic, Shepody, Snowden) and one European variety (Bintje) was comprised. For the development of sequence data for SolCAP, next generation sequence of the transcriptome was used for the cultivars Atlantic, Premier Russet and Snowden also these were collected from the different databases at public level for the varieties like Bintje, Shepody, Kennebec (Hamilton et al., 2011). SolCAP Potato Array helped in a genotyping of a bi-parental tetraploid mapping population (Hackett et al., 2013). First application of SolCAP SNP array, as a pilot study was conducted on European potato germplasm Stich et al., (2013), in which they tested the population structure (Q) and linkage disequilibrium (LD) of 36 different tetraploid cultivars and eight diploid potato clones.

For the analysis of large amounts of SNPs per genotype customized arrays are used (e.g. Hamilton et al., 2011). The marker loci for array were designed in such a way, that 36% of the markers are in candidate gene of interest and 6% are from the previously marked

markers. Additional number of markers that counts 57% in coding region was selected so that maximum coverage to the potato genome can be given (Felcher et al., 2012). In 2012, Felcher et al., elaborated the design of array as well as explained the incorporation of two linkage maps of diploid species with potato genome sequence. Also, with SolCAP SNP potato array tetraploid bi-parental mapping population was genotyped (Hackett et al., 2013).

2.6 Association Mapping

Association mapping has now become an established way to recognize quantitative trait loci (QTL) which is measurable for alterations for desirable characteristics, integral to classic QTL mapping, because of the improvements in the technology molecular marker and in statistical techniques have fashioned association mapping technique approachable as well as economical even for plant breeders (Zhu et al., 2008). Two vital benefits of association mapping are (1) Collecting the cultivars that are related to each other distantly and material for breeding contains all allelic diversity that are relevant and gives the result in more generic way, and (2) dense resolutions while mapping can be achieved as the events of meiotic recombination that are sampled are more distinguished to a mapping population that is biparental segregating (Jannink and Walsh, 2002; Gaut and Long, 2003; Flint-Garcia et al., 2003).

Association mapping just from the onset is a well-known research technique that is being used both for human and animal genetics. First area of Association mapping studies was the genetics of the human disease and gained success as well (Lander and Schork, 1994; Jorde, 2000; Carlson et al., 2004). While dealing with the genetics of animals, principal business was the linkage disequilibrium arrangements among breeding populations for conclusions that to what range LD holds and the marker density needed to map the genes in a very fine way, for example, in cattle (Farnir et al., 2000), sheep (McRae et al., 2002) and pig (Nsengimana et al., 2004). While dealing with plants, nevertheless, few number of studies have been published until now specifically on association mapping, other than the crops like *Arabidopsis thaliana* (Nordborg et al., 2002; Hagenblad and Nordborg, 2002), rice (Lu et al., 2005; Garriss et al., 2003; Semon et al., 2005) and maize (Tenaillon et al., 2001; Ching et al., 2002; Parisseaux and Bernardo, 2004; Palaisa et al., 2003, 2004; Remington et al., 2001; Rafalski and Morgante, 2004), association mapping is achieving significance in these

crops because of the availability of high density marker system as well as the genome sequences of the relevant crop. Other crops have also been examined for LD design and for association. For barley crop, Kraakman et al., (2004, 2006) conducted association mapping for morphological, agronomical as well as the traits that are related to the resistances. Candidate gene approach was adapted to analyze Conifers to anatomize the traits that are complex in nature (Neale and Savolainen, 2004). In the crop of grapevine pattern of LD was described by Barnaud et al., (2006). In *Lolium perenne*, Sköt, (2005) realized some associations for date to heading. LD patterns were analyzed redistributing SNPs in soybean (Zhu *et al.*, 2003), Amplified Fragment Length Polymorphisms in sugar beet (Kraft et al., 2000), also Restriction Fragment Length Polymorphisms in sugarcane (Jannoo et al., 1999). In Wheat, size of kernel and quality of milling were fine mapped practicing association mapping technique (Breseghello and Sorrells, 2006). Recently, comprehensive survey on association mapping studies and the condition of this technique in plants has been released (Gupta et al., 2005).

Linkage mapping can be explained as the association of the traits that are phenotypic in nature with molecular markers in a mapping population. Main purpose of linkage mapping is to find out the regions in the genome that can translate phenotypic variations in any trait in which one is interested, and the consequent description of promising original genes present in specific domain. QTLs are basically the domains of chromosomes that are connected physically to the allele of molecular marker. QTL and that specific marker allele are moving in a composed form. Fundamental quantitative trait, which is widely distributed over the phenotype, may be situated on chromosomes (Gebhardt et al., 2005). Various type of mapping populations can be used for linkage analysis (Collard et al., 2005). Once the mapping populations entrenched, Genotyping with segregating molecular markers are performed and phenotyped for any trait of interest. Molecular marker data helps in the formation of a linkage map and marker trait associations are used to find out the QTLs.

Due to highly heterozygous nature of potatoes diploid potatoes are mainly being used for conducting QTL analysis. Many QTL studies have been conducted that compromise with resistance against root cyst nematodes (Kreike et al., 1994), stresses that are biotic in nature (Li et al., 1998) as well as abiotic stresses like tolerance against drought (Anithakumari et al., 2011). Moreover, output and the characteristics that are related to the quality of the crop, like starch content, yield (Schafer-Pregl et al., 1998), specific

gravity (Freyre and Douches, 1994), discoloration due to enzymatic activities (Werij et al., 2007) and cold sweetening (Menendez et al., 2002) were also examined using QTL mapping technique.

Even though linkage mapping of tetraploid potato is not so simple as it is for diploid potato, yet some successful examples are there that have been conducted with tetraploid potato, like for the studies related to the resistance of late blight disease (Meyer et al., 1998; Bradshaw et al., 1998; Li et al., 1998). Bradshaw et al., (2008) while working on the traits like yield, agronomic traits and tuber quality traits of tetraploid mapping population of full-sib family mapped 16 QTLs. Further studies were conducted by Van Eck (2007) as well as by Milbourne et al., (2007). On other hand, for mapping approach that deals with family-based linkage, association mapping is a process to find out the trait-marker associations even in a population that is slidely associated to each other. Association mapping takes the favor of linkage disequilibrium as well as historical meiotic recombinations (Flint-Garcia et al., 2003).

Linkage disequilibrium (LD) is the base for association mapping. Linkage disequilibrium (LD) is described as the linkage among two alleles in a mapping population on non-random basis (Flint-Garcia et al., 2003). This term stands mostly for the position on a specific gene that is adjacent one another present on the same chromosome. Still, linkage disequilibrium can also happen among the alleles that are not sharing the same chromosome (Flint-Garcia et al., 2003). Concerning LD extent in tetraploid potato different beliefs are there. D'hoop et al., (2010) stated range of 5 cM for genome wide LD. Stich et al., (2013) advised a linkage decay within each 275 bp. Utilization of LD is the basis of association mapping (Soto-Cerda and Cloutier, 2012), where quantitative trait locus as well as marker that are associated are in linkage disequilibrium (LD) and or in the ideal case they are physically linked (Gebhardt, 2013).

For association mapping generally two mapping procedures are being practiced that are Association of Candidate Gene and the second one is Whole Genome Scan, that is also known as Genome-Wide Association Study. In the candidate gene method, hypothesis is tested 'either there is a correlation among the candidate gene and the trait on interest'. e.g. someone can inquire either there is a relationship in a diverse germplasm collection of maize between phytoene synthase sequence alleles of DNA and seeds carotenoid

content (Palaisa et al., 2003; Pozniak et al., 2007; Harjes et al., 2008). This tactic adopts good knowhow among biochemistry and trait genetics, and numerous genes may remain out of attention. Therefore, if there is no detailed knowledge of the biochemical pathway of interest, including the genes that are regulatory in nature, genome scan, defined in next paragraph, is a healthier opinion to deal with.

Genome scan comprises checking for association almost all the genome segments, by genotyping compactly dispersed loci for genetic marker layering over all chromosomes (Figure 1). There is a simple hypothesis under consideration ‘one or more than one locus is being considered fundamental for the trait or in association with fundamental locus’. Association candidate gene, that undertakes some knowhow of the trait genetics, possibly be understood as a subset of a more genome scan approach (Rafalski, 2010).

QTL and genetic studies in potato crop have mainly been completed using diploid populations, e.g. resistance against leaf blight QTLs have been spotted in diploid *S. tuberosum* (Visker et al., 2005), in a diploid *S. phureja* × *S. stenotomum* hybrid population (Simko et al., 2006; Costanzo et al., 2005), in a di-haploid clone (Bradshaw et al., 2006), in diploid *S. vernei* (Sørensen et al., 2006) and in diploid *S. microdontum* (Bisognin et al., 2005) and QTLs for cold sweetening of potato have been plotted through Menendez et al., (2002) also potato of diploid species. Currently, tetraploid potato populations are being practiced for these kinds of studies as well. Simko with his team mapped resistance against Verticillium resistance genes in a population of tetraploid potatoes using various methods of association mapping, like haplotype association, a whole genome approach and candidate genes (Simko et al., 2004a, b). With a restricted number of embattled markers, Bormann et al., (2004) marked QTLs for maturity modified late blight resistance in tetraploid potato. However, the QTL studies in the tetraploid mapping population 12601ab1 × Stirling are unique in representing the intricacy of map creation and QTL analysis (Bradshaw et al., 2004; Bryan et al., 2004).

2.7 Association Studies for Potato

Until now, associations between marker and trait have been observed in potato for the resistance of disease (Malosetti et al., 2007; Sattarzadeh et al., 2006; Kasai et al., 2000; Gebhardt et al., 2004; Song et al., 2005; Flis et al., 2005; Pajerowska-Mukhtar et al.,

2009; Gebhardt et al., 2001; Simko et al., 2004) and quality of tuber traits (D'hoop et al., 2008; Li et al., 2005, 2008). Potato Virus Y and root cyst nematode *Globodera pallida* were among the first genes to be restrained to a single chromosomal position in mapping populations for experiments. After that widely associated cultivars with identified phenotypes that had resistance were tested to find out marker alleles linked to resistance (Witek et al., 2006; Kasai et al., 2000; Song et al., 2005; Sattarzadeh et al., 2006; Flis et al., 2005). These understandings characterize the first case in which highly indicative DNA markers were recognized that are appropriate for breeding applications. Simko with his researchers in (2004) assessed 137 North American populations of tetraploid potato cultivars and advanced breeding lines for quantitative resistance to Verticillium wilt (*Verticillium dahliae*). They were then benefited with the detail that tomato has been found to have *Ve* resistance genes that had been mapped (Kawchuk et al., 2001; Diwan et al., 1999), to recognize orthologous locus *StVe* on potato chromosome IX. Quantitative resistance to *Verticillium dahliae* was found associated to SSR allele that is strictly linked to *StVe* (1.5 cM) (Simko et al., 2004). Many diploid and tetraploid mapping populations were marked for *R* genes and QTL for late blight resistance (*Phytophthora infestans* (Simko et al., 2007; Gebhardt and Valkonen, 2001).

In association mapping studies, candidate gene technique was extended which was aimed to find the DNA variations that are related to field resistance to late blight disease that was not being dealt with the late plant maturity (Pajerowska-Mukhtar et al., 2009). 192 tetraploid breeding potato clones were grown to check late blight field resistance as well as for maturity of plants and then was genotyped for 230 SNPs at 24 different candidate loci. 09 SNPs were found meaningfully ($P < 0.001$) related with maturity corrected resistance, which mutually elucidated 50% of the genetic variance of this trait. In the association study conducted by Malosetti and his team in 2007, 204 tetraploid potato cultivars were genotyped for profiling of NBS. Association tests with present late blight resistance phenotypic data exposed between 2 and 30 trait marker associations, depending on the association model applied. Two NBS markers with unidentified map position were steadily associated with resistance to late blight. Polygenic inheritance is present almost in tubers phenotypic character (van Eck, 2007) as well as the common alleles can result in genotypic changes. Furthermore, Carbohydrate linked traits like content of sugar and starch content can assist as role for the candidate gene approach for the identification of QTL in potato, as metabolism of the carbohydrate has deeply been

measured at the different physiological, biochemical and molecular levels (Isherwood, 1973; Hofius and Börnke, 2007).

2.8 Analysis of Population structure to prevent false-positives:

The genotypes of a potato that are being cultivated now a days are the collection of the individuals that are distinctly linked to each other (Gebhardt et al., 2005). Therefore, a promising influence towards relationship during the analysis which are conducted statistically, which aids that some interested characteristics can be connected to a gene pool (Flint-Garcia et al., 2003). Information regarding extent of relationship among the mapping population genotypes performs a vital part in association mapping to escape from false positives in the population. Although marker might not be connected to a QTL, a significant chance of detecting a substantial association that can only happen due to the genetic relationship among the participants might be there (Pritchard et al., 2000).

Based on genetic markers, there are various choices to approach population structure in potato crop. The two options that are principal coordinate analysis and principal component analysis emerging from factor analysis approach (Urbany et al., 2011; D'hoop et al., 2010; Pajerowska-Mukhtar et al., 2009), where molecular marker data is processed after having the genotyping information. On the other hand, the data of marker is accessed using Bayesian clustering method, put into the action in the “Structure” software (Pritchard et al., 2000). Many studies in potato research by using this technique (Pajerowska-Mukhtar et al., 2009; Simko et al., 2006; Simko, 2004; D'hoop et al., 2010; Li et al., 2008). Population structure can be accessed by the help of hierarchical clustering and AMOVA (Analysis of Molecular Variance) (D'hoop et al., 2010).

2.9 Applications of Association Mapping

Genetic variations that elaborates the variations of complex traits in plants can be detected with the help of association mapping, such as in maize, wheat, barley, rice, perennial ryegrass, Arabidopsis, rapeseed and sugar beet association mapping studies have successfully been administered (Aranzana et al., 2005; Breseghello and Sorrells, 2006; Wilson et al., 2004; Cockram et al., 2008; Skot et al., 2005; Huang et al., 2011;

Stich and Melchinger, 2009). Gebhardt et al., (2004) published the first report in tetraploid potato germplasm, who collected and examined a massed set of 600 cultivars of potato to find out trait marker associations for the resistance against the disease of late blight and maturity based on historic recombination events. Additionally, association mapping work that is based on the candidate gene approach, pursued for the resistance against *Phytophthora infestans* (Pajerowska-Mukhtar et al., 2009; Malosetti et al., 2007). Other illustration regarding studies of association mapping involves some traits regarding tuber quality like tuber starch content, yield of tuber, tuber starch yield and chip quality of tuber (Li et al., 2008, 2005; Fischer et al., 2013). Likewise, susceptibility to tuber bruising, shape of the tuber and maturity of the plants were also considered in tetraploid potato population by association mapping (Urbany et al., 2011).

Genome wide association studies provide us broader way to have a look at marker-trait associations. It was D'hoop et al., (2008), who firstly used association mapping for the first time, although the marker number was rather low in his studies. Uitdewilligen et al., (2013) gave additional example for genome-wide association mapping with small genotype panel.

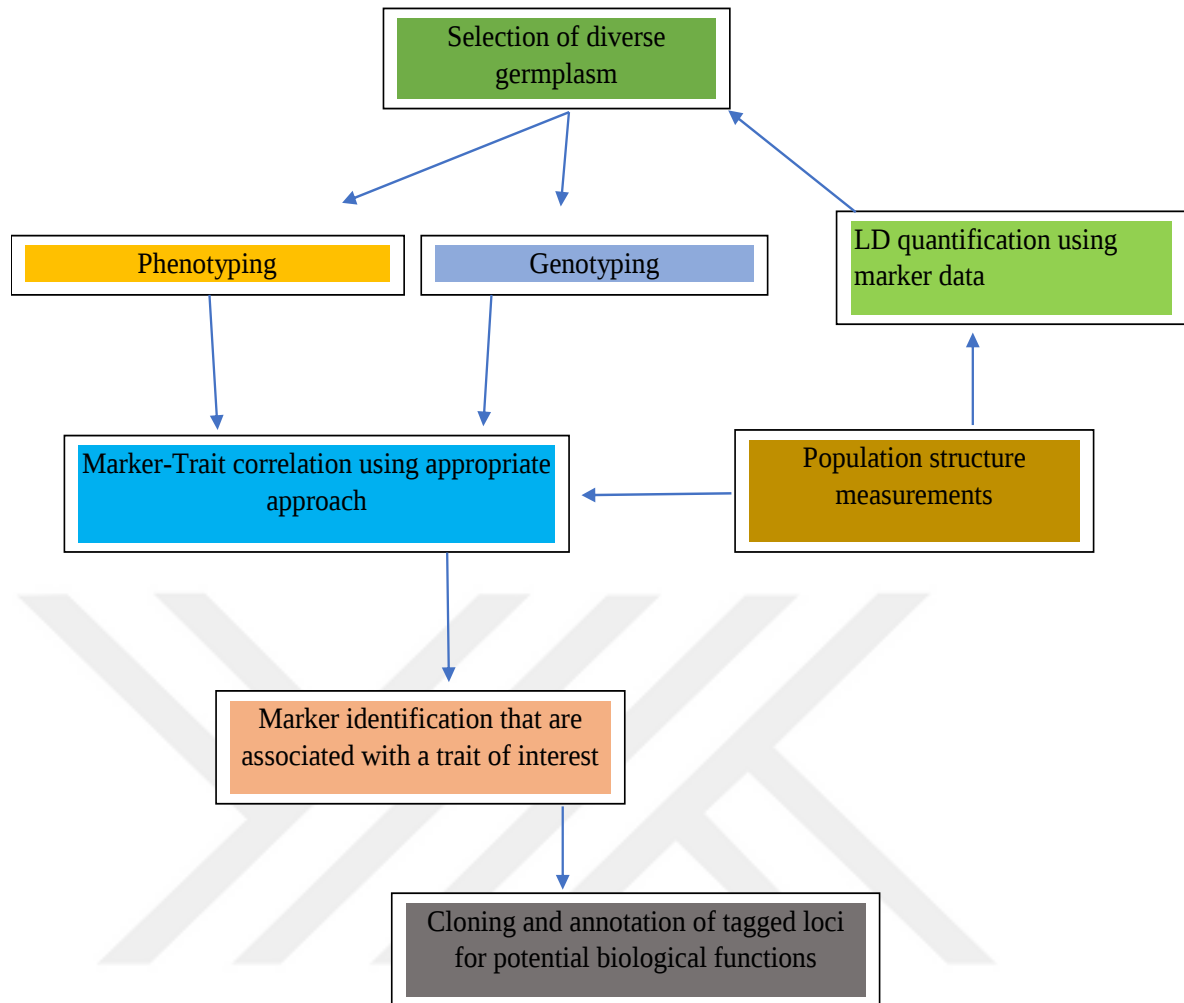


Figure 2.1. Association mapping scheme to tag a gene of interest using different kind of germplasm

2.10 Advantages of Association Mapping over Linkage Mapping

Association mapping have three main advantages over linkage mapping as per Flint-Garcia et al., (2003). Foremost, in association mapping the mapping resolution is superior since it is using higher number of meiotic incidents; however linkage mapping considers the single meiotic generation for recombination normally (Gebhardt, 2007). Nonetheless, while dealing with the potatoes, this benefit is not such momentous, as Gebhardt et al., (2004) came to know that comparably less meiotic generations can cause the isolation of individual genotypes. This might be because of the reason that potato is being propagated with clonal propagation where the meiotic generation is preserved.

Second, an increased count of alleles can be found by using association mapping technique. In a population that is segregating, utmost number of various alleles that are observed at the single locus in diploid linkage mapping population offspring are four while for mapping population of tetraploid linkage it counts eight. For a massed populace of almost 200 tetraploid genotypes, highest count for various alleles at a single locus is 800 theoretically. On account of a decreased statistical competence, trait-marker associations of very infrequent alleles cannot be revealed. That's why; association mapping is reliable to detect the typical variations (Flint-Garcia et al., 2003). Third, markers might be practiced at once to any breeding program. Identified markers are unswervingly and widely suited to the mapping population that entails of suitable material for breeding (Stich and Melchinger, 2010; Li et al., 2013).

2.11 Approach to Anatomize the Quantitative Traits

Even with expansion of unique high-throughput methods for genotyping the expenditure for high-throughput genotyping have lowered to the specific level for example, SolCAP Potato Array for potato species, still it is very costly methods specially for the breeders for the analysis of the germplasm completely. For less number of genotypes to be analyzed combining strategies have been developed. For plant studies a prevailing approach is analysis using analysis of bulked segregants and for the studies related to human disease, case-control study is being practiced.

2.11.1 Analysis using bulked segregants

Segregating bi-parental population is necessary while using bulk segregant analysis (Collard et al., 2005). Two groups are bulked with the genotypes with extremely different phenotypes and then at genetic level they are compared for lurking genetics labeling. Detecting markers are the main theme of bulked segregant analysis that is closely connected to QTL (Meksem et al., 1995; Collard et al., 2005). Merging strategy can play as an alternate for the identification of markers in a bulked segregant analysis for tagging of QTL (Collard et al., 2005) it can also be used effectively for discovering the candidate gene that have some influence on trait of interest (Kloosterman et al., 2010). Bulked segregant analysis has been applied for the analysis of complex traits on a large group of crop studies, like in maize, barley, rice and also in grapevine (Takagi et al., 2013; Donald et al., 2002; Quarrie et al., 1999; Zhang et al., 2009; Chen et al.,

2011). Analysis of bulk segregant has also been considered for the fine mapping genes of polyploid wheat aside from these diploid crops (Trick et al., 2012). Qualitative traits have been tested in potato using bulked segregant analysis (Meksem et al., 1995; Li et al., 1998), even quantitative traits were examined in diploid as well as in tetraploid genetic background. Ballvora et al., (2011) analyzed half-sib families of two tetraploid species while working with potato wart disease resistance. Likewise, Kloosterman et al., (2010) analyzed appropriateness of bulked segregant analysis for mapping population of diploid species and proposed that by profiling experiment administrating a bulked segregant analysis to find contender genes for tuber fresh quality, cooking type as well as free methionine content.

2.11.2 Case control studies

Case-control study is used for dissecting the genetic background of complex traits phenotypically different groups are compared. The theme behind this is extensively used for human disease studies (Balding, 2006) and that is why relatedness of phenotypic groups are by descent, instead selected from mapping population of bi-parental species as being accomplished in the bulked segregant analysis. Purpose is getting the concept of the similar disease, hazard factor involved and the genetic contextual of the recognized variations by screening these pools (Balding, 2006). Huang et al., (2011) adopted case-control study over genome wide association mapping studies to maximize the options to find new trait marker relations in maize. In potato, using comparative proteomics, this technique has favorably been adopted to identify candidate genes. Urbany et al., (2011) found the unique candidate genes by comparing the protein expression after assembling two genetic pools of ten genotypes each that differed in tuber bruising susceptibility. Later on, the obtained candidate genes were analysed through association mapping using potato cultivars (Urbany et al., 2011). Likewise, Fischer et al., (2013) comparative proteomics was practiced for the identification of unique candidate genes that are linked to cold-induced sweetening of potato cultivar. Association of candidate gene proved that the variations in DNA sequence interpreted the phenotypic variation among cold sweetening in potato tubers.

Concluding all this we can say that although the numbers of research papers using association mapping technique in plants are not much enough as compared to animal or human genetics, an obvious flow towards the increased number of crop species that are

being examined for association mapping has been accomplished with favorable outcome in the recent few years.



CHAPTER III

MATERIALS AND METHODS

Two hundred thirty-seven (237) potato genotypes having different origin were used in the thesis (Table 3.1). Most of the genotypes (194) were introduced from International Potato Center (CIP), Peru while 10 of them from Hungary, 2 of them from Japan. 24 breeding lines from our breeding program and 5 standard cultivars (Agria, Desiree, Granola, Melody and Russet Burbank) grown in Turkey were used. The field experiments were conducted to derive phenotypic data in 2016 and 2017 at the Experimental Farm of Potato Research Institute Niğde (Figure 3.1 and 3.2). The genotypes were planted in Augmented Design (Petersen, 1985) with five standard cultivars. Experiment was planted on 23rd May in 2016 and 12th of May in 2017 and was harvested on 26th of October in 2016 and 6th of October in 2017. Each experimental plot was consisted of two rows of 75 cm apart and 6 m long, and twenty tubers were planted in each row with 30 cm distance. Standard potato production practices were followed during growing period in both years.

Table 3.1. List of genotypes studied

No	Genotype	No	Genotype	No	Genotype	No	Genotype
1	CIP300046.22	61	CIP388972.22	121	CIP396012.266	181	CIP398208.33
2	CIP300048.12	62	CIP389468.3	122	CIP396038.101	182	CIP398208.620
3	CIP300054.29	63	CIP389746.2	123	CIP396038.105	183	CIP398208.670
4	CIP300055.32	64	CIP390478.9	124	CIP396063.1	184	CIP399067.22
5	CIP300056.33	65	CIP390637.1	125	CIP396063.16	185	CIP399078.11
6	CIP300063.9	66	CIP390663.8	126	CIP396180.22	186	CIP399085.30
7	CIP300065.4	67	CIP391004.18	127	CIP396268.1	187	CIP694474.16
8	CIP300066.11	68	CIP391046.14	128	CIP396268.9	188	CIP720072
9	CIP300093.14	69	CIP391065.69	129	CIP396269.14	189	CIP800258
10	CIP300099.22	70	CIP391065.81	130	CIP396269.16	190	Konyu-2
11	CIP300101.11	71	CIP391207.2	131	CIP396272.12	191	Konyu-3
12	CIP300135.14	72	CIP391382.18	132	CIP396272.18	192	PANU 01.536
13	CIP300135.3	73	CIP391580.30	133	CIP396272.2	193	PANU 07.258
14	CIP301023.15	74	CIP391583.25	134	CIP396272.21	194	PANU 02.173
15	CIP301024.95	75	CIP391585.179	135	CIP396272.37	195	PANU 03.113

Table 3.1. (Continue) List of genotypes studied

No	Genotype	No	Genotype	No	Genotype	No	Genotype
16	CIP301026.23	76	CIP391585.5	136	CIP396273.48	196	PANU 02.363
17	CIP301029.18	77	CIP391724.1	137	CIP396285.1	197	PANU 08.212
18	CIP301041.26	78	CIP391931.1	138	CIP396287.5	198	PANU 06.62
19	CIP301044.36	79	CIP392025.7	139	CIP396311.1	199	Balatoni Rosza
20	CIP301045.74	80	CIP392634.49	140	CIP397006.18	200	Arony Chipke
21	CIP301055.53	81	CIP392639.34	141	CIP397012.20	201	Demon
22	CIP302428.20	82	CIP392657.171	142	CIP397016.7	202	NOHU 0601.17
23	CIP302476.108	83	CIP392740.4	143	CIP397029.21	203	NOHU 0601.19
24	CIP302498.70	84	CIP392745.7	144	CIP397030.31	204	NOHU 0601.20
25	CIP302499.30	85	CIP392781.1	145	CIP397035.26	205	NOHU 0702.18
26	CIP303381.106	86	CIP392821.1	146	CIP397036.7	206	NOHU 0704.12
27	CIP303381.30	87	CIP392822.3	147	CIP397039.53	207	NOHU 0714.12
28	CIP304345.102	88	CIP392973.48	148	CIP397060.19	208	NOHU 1204.01
29	CIP304347.6	89	CIP393079.24	149	CIP397067.2	209	NOHU 1204.03
30	CIP304349.8	90	CIP393085.5	150	CIP397069.5	210	NOHU 1204.04
31	CIP304350.100	91	CIP393248.55	151	CIP397073.15	211	NOHU 1301.25
32	CIP304350.118	92	CIP393284.39	152	CIP397073.7	212	NOHU 1301.07
33	CIP304350.18	93	CIP393371.164	153	CIP397077.16	213	NOHU 1301.16
34	CIP304350.78	94	CIP393399.7	154	CIP397078.12	214	NOHU 1301.02
35	CIP304350.95	95	CIP393613.2	155	CIP397079.26	215	NOHU 1301.02
36	CIP304351.109	96	CIP393615.6	156	CIP397098.12	216	NOHU 1301.02
37	CIP304351.31	97	CIP393617.1	157	CIP397099.4	217	NOHU 1301.21
38	CIP304366.46	98	CIP393708.31	158	CIP397099.6	218	NOHU 1307.04
39	CIP304369.22	99	CIP394034.65	159	CIP397100.9	219	NOHU 1301.21
40	CIP304371.20	100	CIP394034.7	160	CIP397196.3	220	NOHU 1301.08
41	CIP304371.67	101	CIP394223.9	161	CIP397196.8	221	NOHU 1301.03
42	CIP304383.41	102	CIP394600.52	162	CIP397197.9	222	NOHU 1301.01
43	CIP304383.80	103	CIP394613.32	163	CIP398014.2	223	NOHU 1302.18
44	CIP304387.92	104	CIP394638.3	164	CIP398098.119	224	NOHU 1307.04
45	CIP304394.56	105	CIP394881.8	165	CIP398098.205	225	NOHU 1302.02
46	CIP304399.15	106	CIP394895.7	166	CIP398098.231	226	CIP 37

Table 3.1. (Continue) List of genotypes studied

No	Genotype	No	Genotype	No	Genotype	No	Genotype
47	CIP304399.5	107	CIP395011.2	167	CIP398098.570	227	CIP 38
48	CIP304405.42	108	CIP395017.227	168	CIP398098.65	228	CIP 45
49	CIP304405.47	109	CIP395017.229	169	CIP398180.144	229	CIP 49
50	CIP374080.5	110	CIP395017.242	170	CIP398180.253	230	BANBA
51	CIP377744.1	111	CIP395123.6	171	CIP398180.289	231	Jelly
52	CIP379706.27	112	CIP395186.6	172	CIP398180.612	232	UNICA
53	CIP380496.6	113	CIP395196.4	173	CIP398190.112	233	Agria (st)
54	CIP381381.13	114	CIP395432.51	174	CIP398190.404	234	Desiree (st)
55	CIP381381.9	115	CIP395434.1	175	CIP398190.571	235	Granola (st)
56	CIP384866.5	116	CIP395436.8	176	CIP398190.615	236	Melody (st)
57	CIP385499.11	117	CIP395445.16	177	CIP398193.158	237	Russet Burbank (st)
58	CIP385558.2	118	CIP396004.337	178	CIP398193.650		
59	CIP385561.124	119	CIP396009.258	179	CIP398208.219		
60	CIP388611.22	120	CIP397012.22	180	CIP398208.29		

NOHU= Nigde Omer Halisdemir University, CIP = International potato center, PANU = Pannonia university Hungary, Konyu = Hokaido University Japan, BANBA is variety from U. K. and Jelly is a Dutch variety

First year and second year research was carried out having 237 different potato lines. A 20 different agronomic and morphological traits were studied both phenotypically as well as genotypically.

**Figure 3.1.** Crop of growing season 2016



Figure 3.2. Crop for the growing season of 2017

3.1 Plant Traits

Following traits were handled while phenotyping:

3.1.1 Growth habit (GH)

Growth Habit of the specific genotype was observed by following the Potato Descriptor from Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA. According to the descriptor these classes were divided in 3 classes i.e. 1- Tall, 2- Low, 3- Medium

3.1.2 Branching habit (BH)

Branching habit was recorded by following the potato descriptor (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor these classes were divided in 3 classes i.e. 1- Upright, 2- Semi Upright, 3- Spreading

3.1.3 Plant height (PH)

Ten plants were selected at random from each genotype. Their heights were measured in cm with measuring tape from base to tip and then were averaged.

3.1.4 Stem thickness (ST)

Stem thickness of ten plants were taken from the main stem near to the soil by the help of digital compass in mm and was averaged.

3.1.5 Leaves' shape (LS)

Leaves' shape was recorded by using potato descriptor (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor these classes were divided in 3 classes i.e. 1- Closed leaf structure, 2- Intermediate leaf structure, 3- Open Leaf Structure



Figure 3.3. Different leaves shapes in potato crop. Closed leaf structure (a), Intermediate leaf structure (b), Open Leaf Structure (c)

3.1.6 Number of leaflets (NL)

Number of leaflets per leaf of 10 different plants was counted manually and was averaged.

3.1.7 Maturity (M)

Maturity was recorded manually by the growth cycle of the plants and then scored according to the potato descriptor (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor these classes were divided in 5 classes i.e.; 1- Very Late, 2- Late, 3- Intermediate, 4- Early 5- Very Early.

3.2 Flower Traits

3.2.1 Flower color (FC)

Flower color was observed and written as per appearance. Flower color was given the numbers like White = 1, Purple = 2, Light Purple = 3, Very light purple = 4, Whitish purple = 5, Bluish purple = 6, Dark purple = 7, Shaded yellow purple = 8, Yellowish purple = 9 and Bluish white = 10.



Figure 3.4. Different flower colors in potato crop from different genotype lines

3.2.2 Pistil length (PL)

Pistil length of flowers from 10 different plants were taken by digital compass and then averaged.

3.2.3 Stamen length (SL)

Stamen length of flowers from 10 different plants were taken by digital compass and then averaged.

3.2.4 Pistil length above stamen (PLAS)

Pistil length above stamen of flowers from 10 different plants were taken by digital compass and then averaged.

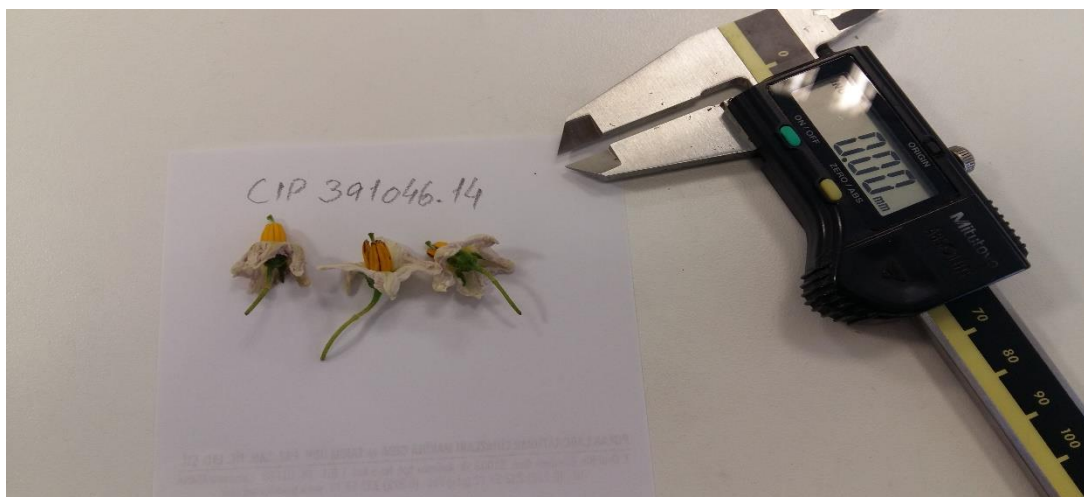


Figure 3.5. Digital Compass to measure the traits related to Stamen and Pistil length in potato flower

3.3 Tuber Traits

3.3.1 Tuber skin color (TSC)

Tuber skin color was recorded by following potato descriptors (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor these classes were divided in 6 classes i.e. 1- Yellow, 2- Red, 3- Red colored, 4- Blue, 5- Reddish Brown, 6- Blue colored.

3.3.2 Number of tubers per Plant (NTP)

Number of tubers was counted by from 10 sample plants from each genotype and was averaged.

3.3.3 Tuber shape (TS)

Tuber shape was assessed by following the potato descriptors (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor these classes were divided in 6 classes i.e. 1- Round, 2- Short Oval, 3- Oval, 4- Long Oval, 5- Long, 6- Very Long.

3.3.4 Average tuber size (TSz)

Tuber size was assessed by following the potato descriptors (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor these classes were divided in 5 classes i.e. 1- Very small, 2- Small, 3- Medium, 4- Large, 5- Very large.

3.3.5 Tuber flesh color (TFC)

Tuber flesh color was also taken by following different potato descriptors (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor it was divided in 7 classes i.e. 1- White, 2- Cream, 3- Light yellow, 4- medium yellow, 5- Dark yellow, 6- Red, 7- Blue

3.3.6 Tuber skin texture (TST)

Tuber Skin texture was measured by following the different potato descriptors (Potato Guidelines by International Union for Protection of New Varieties of Plants in GENEVA). According to the descriptor it was divided in 2 classes i.e. 1- Smooth, 2- Rough

3.3.7 Yield:

Tuber Yield was taken after harvesting in grams. Tubers from a single plant was taken and weighted for the yield per plant in grams for every plant in the plot.

3.4 Tuber Quality Traits

3.4.1 Specific gravity (SG)

Specific gravity of the genotype was measured by using Martin Lishmans's Digital Potato Hydrometer following providers' instructions

3.4.2 Dry matter content (DM)

Dry matter content of the genotype was measured by using Martin Lishmans's Digital Potato Hydrometer following providers' instructions.



Figure 3.6. Martin Lishmans's digital potato Hydrometer used to measure specific gravity and dry matter content of potato crop

3.5 DNA Extraction and Genotyping

Genomic DNA was previously extracted using the GeneJET Plant Genomic DNA Purification mini kit (Thermoscientific) according to the supplier's instructions as part of a project supported by TUBİTAK, Project No: 115O406. Quantity and quality of DNA were determined using the BioSpec-nano (Shimadzu) spectrophotometer and standard electrophoresis on 1.2% agarose gels, respectively. Within the same project, SNP genotyping was performed for 237 potato genotypes using the SolCAP Potato genotyping array (Hamilton et al., 2011) by Global Genetik Teknolojileri ve Sağlık Ltd. Şti. Istanbul, Turkey (<http://www.globalgenetik.com>). Genotyping was performed using the array available in the Infinium 12K potato SNP array. The array was read in the Illumina HiScan SQ system. The software Genome Studio version diploids and

polyploids was used to assign the genotype to each locus; five possible genotypes (AAAA, AAAB, AABB, AB BB or BBBB) in tetraploid. The data we got after genotyping was in tetraploid form and to be able to use that in TASSEL we had to transform our data in diploid form as per following criteria,

AAAA----- AA
AAAB, AABB, AB BB----- AB
BBBB----- BB

Total number of markers that we got after SolCAP 12K array was 12,720 markers and out of which 2,911 markers 22.88% were removed as being monomorphic in nature. So, the markers used in association study were totally polymorphic in nature. Out of 9,809 polymorphic markers 6,224 remained unassociated to any of the chromosome in the population and totally 3,585 markers got attached to different chromosomes with an average of 299 markers per chromosome. Best Linear Unbiased Estimator was measured for the data sets of 2016 and 2017. Best linear unbiased estimator is actually the best linear unbiased estimator (BLUE) of the coefficients is given by the ordinary least squares estimator, provided it exists. Here "best" means giving the lowest variance of the estimate, as compared to other unbiased, linear estimators. The errors do not need to be normal, nor do they need to be independent and identically distributed (only uncorrelated with mean zero and homoscedastic with finite variance). The requirement that the estimator be unbiased cannot be dropped, since biased estimators exist with lower variance. To calculate the best linear unbiased estimates (BLUEs) of each genotype, we assumed fixed intercept and genotypic effects, whereas all other effects in the aforementioned model remained random. Quantile-Quantile plots were drawn to see that the model we are using is stringent enough to control population stratification or false positives among our genotypes. Q-Q plot actually is a graphical tool to help us assess if a set of data plausibly came from some theoretical distribution such as a Normal or exponential. A Q-Q plot is a scatterplot created by plotting two sets of quantiles against one another. If both sets of quantiles came from the same distribution, we should see the points forming a line that's roughly straight. It's just a visual check, not an air-tight proof, so it is somewhat subjective. But it allows us to see at-a-glance if our assumption is plausible and if not, how the assumption is violated and what data points contribute to the violation.

3.6 Population Structure and Association Mapping

Augmented analysis was performed to get the estimated values based on the standards by using statistical software SAS. Population structure and genetic similarity (kinship) was analyzed. Population structure was analyzed with Bayes Bayesian clustering approach by using STRUCTURE software (Pritchard et al., 2000). Polymorphic information content for the population under study was calculated using the following formula,

$$PIC = 1 - \sum_{i=1}^n p_i^2 \quad (3.1)$$

Where ‘n’ denotes the total number of alleles and ‘p’ refers to the frequency of the ‘i’th allele at a genetic locus in different genotypes. PIC value for the genotypes that we used was found 0.352 which suggests that our population is diverse enough to be used in association mapping. Rogers distance (RD) of the population was calculated according to Rogers (1972). Based on the estimated RD values, principal coordinate analysis (Gower 1966) was performed. Therefore, relationship between clusters was predicted. Genetic diversity of 237 potato genotypes was calculated according to Nei, (1987). The LD decay was estimated using a combination of SNP markers in significant correlation with a threshold of r^2 that corresponded to 90th percentile of pairwise correlations of each population. Linkage disequilibrium was estimated for all SNPs with D’, r^2 or chi-square, based on allele frequencies (Hill and Robertson, 1968; Achenbach et al., 2009). Trait-marker association was estimated using General linear model as well as using mixed linear model (Zhang *et al.* 2010). For association mapping studies, R (R Core Team (2017) and TASSEL (Bradbury et al., 2007) softwares were used. GWASpoly Package (Rosyara et al., 2016) was used for association studies; q-q man package was used to perform manhattan analysis from the p values obtained from TASSEL. In TASSEL, General Linear Model as well as Mixed Linear model was performed to check the relationship of SNPs to the quantitative traits of the crop as well as for LD analysis. For manhattan plot analysis threshold was set at the level of 5%. There are different threshold that can be a standard. Some of the scientists are putting it on 3%. In that case the threshold line on manhattan plot goes above and the possibility of finding false positives in our data sets remains very less.

3.6.1 General Linear Model (GLM):

Associations between markers and mean phenotypic values were identified using the population membership estimates as covariates to control for population structure. The GLM does not account for kinship as a potential cause of the genotype-phenotype relationship.

A general model was implemented for genome-wide association studies (GWAS) as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{u} \quad (3.2)$$

Where $\mathbf{y}$ is a dependent variable, $\boldsymbol{\beta}$ is a matrix comprising of the parameters that are under study, $\mathbf{u}$ is matrix containing errors, and $\mathbf{X}$ is the matrix of observation on independent variable

3.6.2 Mixed Linear Model (MLM):

It takes account of population structure and kinship in the association analysis. It reduces Type I error due to relatedness and population structure.

A regular linear mixed model was implemented for genome-wide association studies (GWAS) as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e} \quad (3.3)$$

$\mathbf{y}$ is the vector of the BLUEs of each genotype, $\boldsymbol{\beta}$ is a vector containing fixed effects of the genetic marker and the intercept, $\mathbf{u}$ is a vector of random additive genetic effects of each genotype, $\mathbf{X}$ and $\mathbf{Z}$ are the corresponding design matrices and $\mathbf{e}$ is the vector of residuals. In the model, the genetic ($\mathbf{u}$) and the residual ($\mathbf{e}$) effects were assumed random, independent and normally distributed.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Cluster Analysis:

Cluster analysis was performed based of principal coordinate analysis using genotypic data with the statistical software R to see the diversity of our genotypes. Based on the results of principal coordinate analysis we came to know that in our genotypes there are four different types of clusters (Purple, Green, Pink and Blue). Three of them intermingle each other sharing more or less common area showing that they have somewhat similar origin. One of the clusters was entirely separate from all of them having the separate origin. We investigated those genotypes and found that these lines were also from International Potato Center (CIP) Peru but with different structural dimensions. The analyses were conducted by varying the number of possible subpopulations (K) from 1 to 10, with five independent repetitions, assuming an admixture model with correlated allele frequencies and a burn-in of 50.000 and 150.000 iterations.

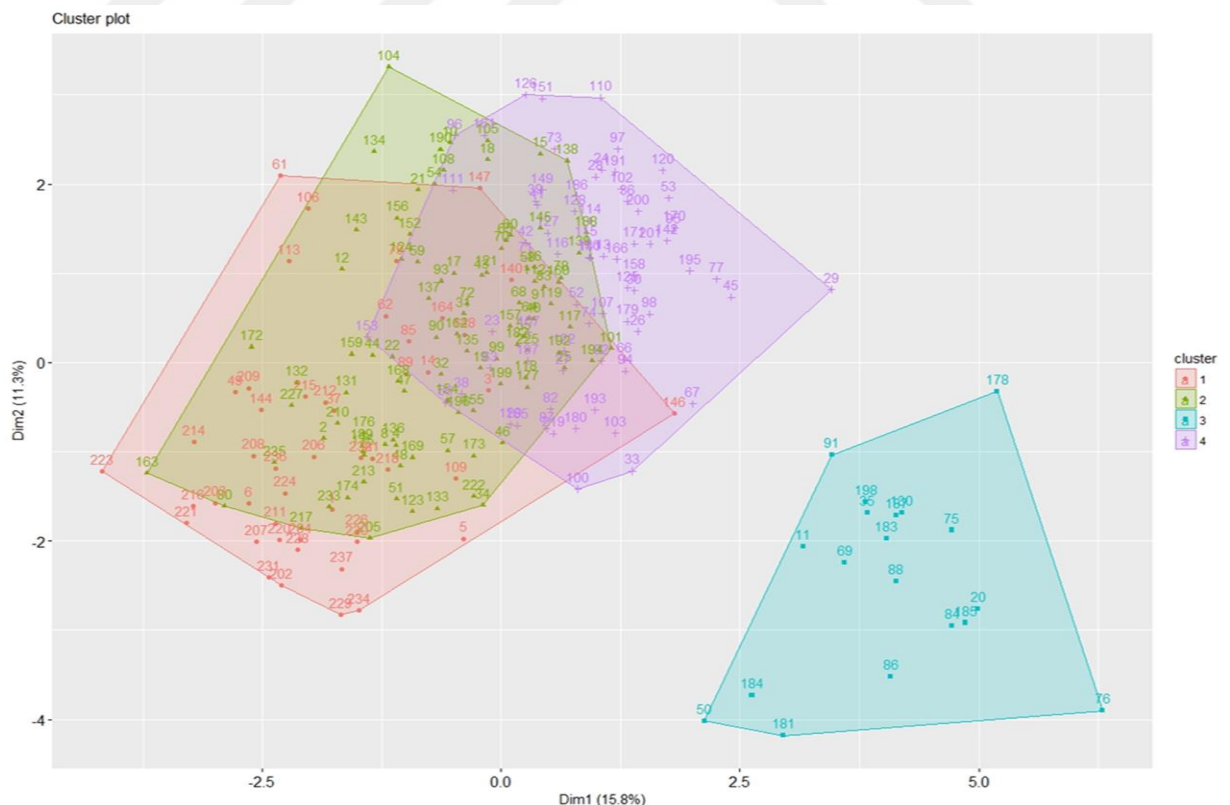


Figure 4.1. Cluster Analysis of the genotypes

Those genotypes that have different cluster from the other three includes

Table.4.1. List of genotypes included in separate cluster of cluster analysis

No.	Genotype	No.	Genotype
1.	CIP 398098.205	11.	CIP 398180.289
2.	CIP 300054.29	12.	CIP 391691.96
3.	CIP 399078.11	13.	CIP 391004.18
4.	CIP 390663.8	14.	CIP 391585.179
5.	CIP 391391.1	15.	CIP 391585.5
6.	CIP 387164.4	16.	CIP 300135.14
7.	CIP 304383.80	17.	CIP 398180.253
8.	CIP 398098.65	18.	CIP 398098.65
9.	CIP 396272.18	19.	CIP 397069.5
10.	CIP 396004.337	20.	CIP 395436.8

4.2 Dendrogram

Based on the kinship analysis taken from TASSEL following neighborhood joining circular tree was formed. This shows how the genotypes are linked to each other. These results also showed the same thing that was represented in the cluster analysis that three of the clusters were like each other and one of them was different from them. In neighborhood joining dendrogram, like in population cluster analysis we found that there were 4 different clusters. Three of them were close and similar to each other but one of that four was showing different characteristics from the rest of three. From these results we came to know that our population had some diversified population to be used for association analysis. From cluster analysis and dendrogram we concluded that there was no genotype that found common in both the tests. They both contained different genotypes in their separated cluster.

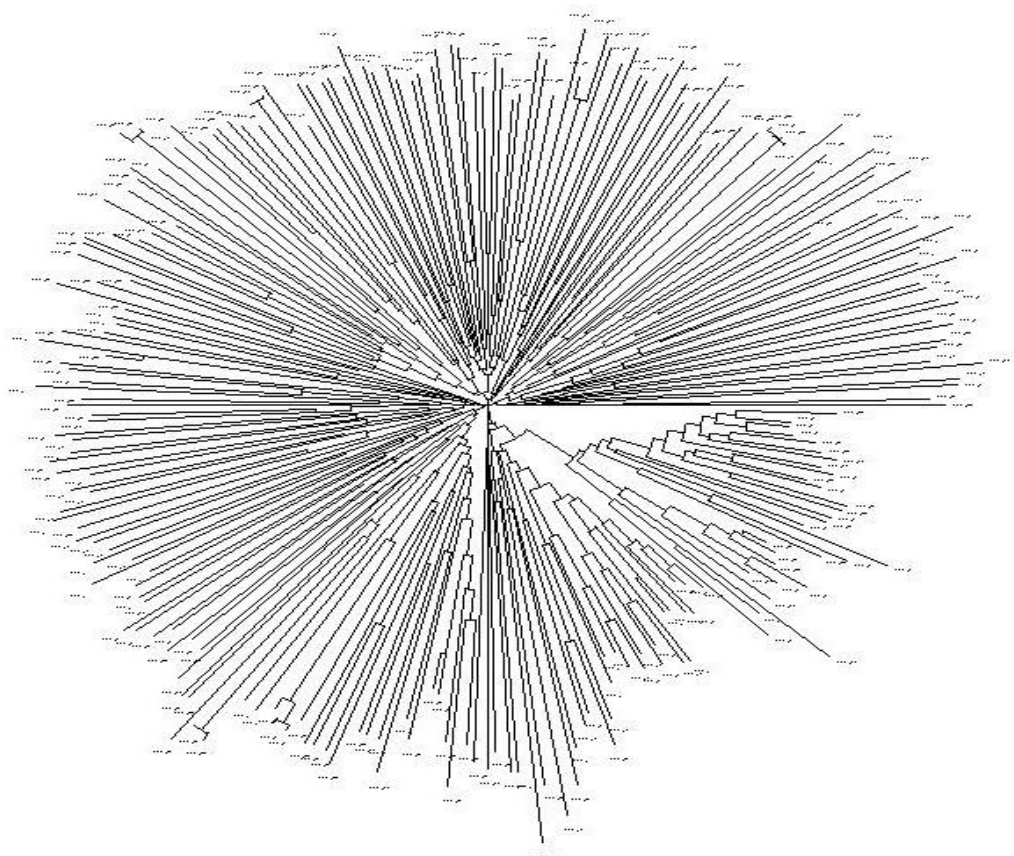


Figure 4.2. Dendrogram of the phenotypes prepared using genotypic data

4.3 Correlation Analysis:

Correlation Analysis was conducted for all the traits to know the relationship of all the traits to each other. Analysis was performed with the statistical software GenStat v18 (VSN International). The genetic correlation has two types: positive correlation, which means the change of the two traits be in the same direction (increase or decrease), and the negative correlation, which means the increase in the first trait combined with a decrease in the second trait (or reverse). The significant correlation level was kept at $p \leq 0.05$. Dry matter content and specific gravity was found to be positively correlated to each other in each data set having r^2 value of exact 1 i.e. 2016, 2017 as well as for BLUE. There was very strong relationship among number of tubers per plant and tuber yield among 2016, 2017 as well as in BLUE readings. Likewise, there was a strong positive relationship among the traits like pistil length with pistil length above stamen ($r^2 = 0.5, 0.75, 0.65$), tuber size and tuber yield ($r^2 = 0.30, 0.50, 0.70$), growth habit and branching pattern ($r^2 = 0.50, 0.60, 0.55$) in all the data set (respectively in 2016, 2017

and BLUE). For the traits like Stamen length and Pistil length they are also in strong positive relationship ($r^2 = 0.70, 0.75, 0.75$) with each other in all the data sets. Some of the traits were even negatively correlated to each other for example for the traits like growth habit and tuber flesh color ($r^2 = -0.25, -0.50, -0.37$). Some of the traits were found having no effect at all with the others for example tuber skin color and tuber skin texture, tuber size and branching pattern, dry matter content and Stamen length don't have any effect on each other.

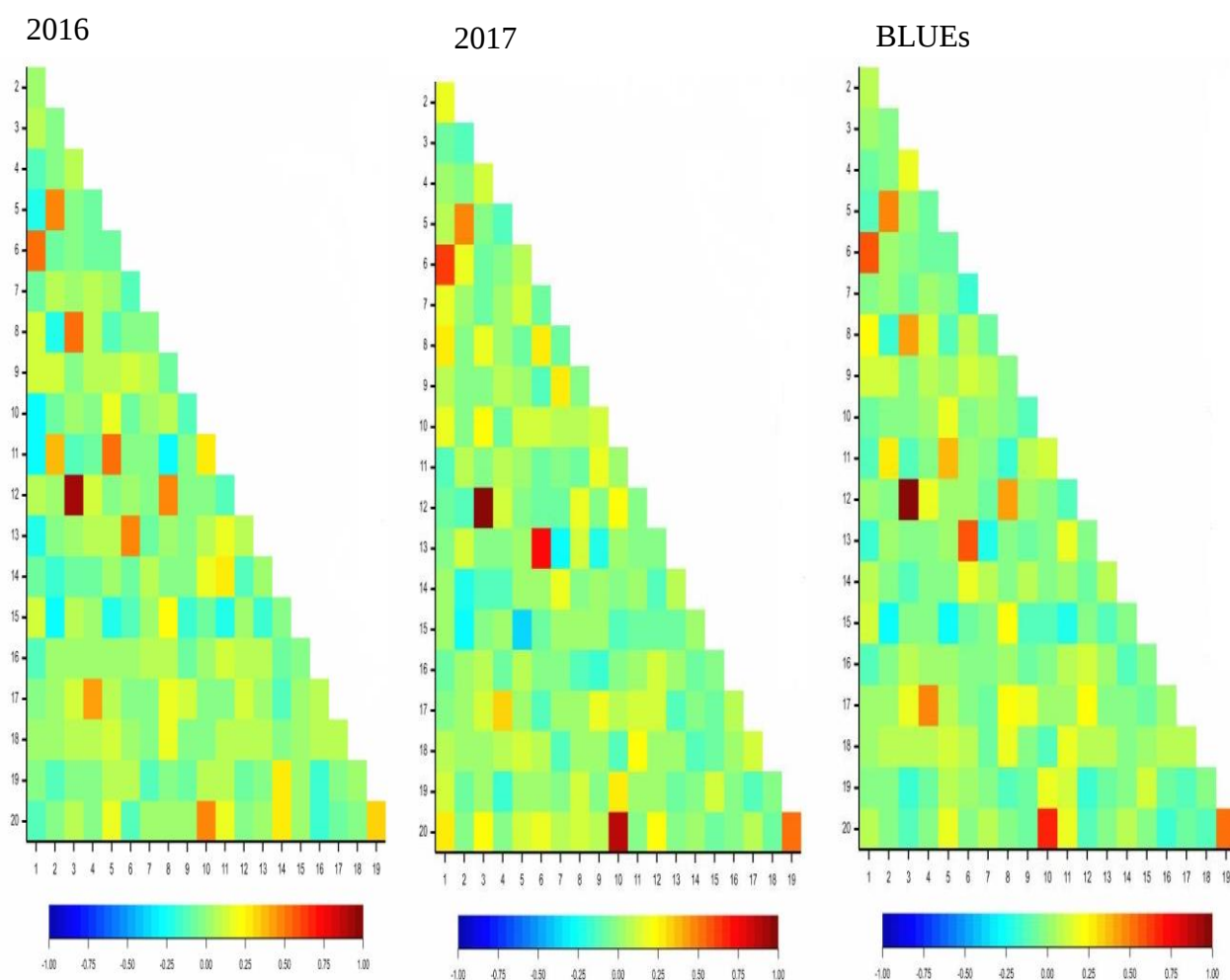


Figure 4.3. Correlation Analysis of the traits for the year 2016, 2017 and BLUE. Colors in the picture shows the correlation intensity

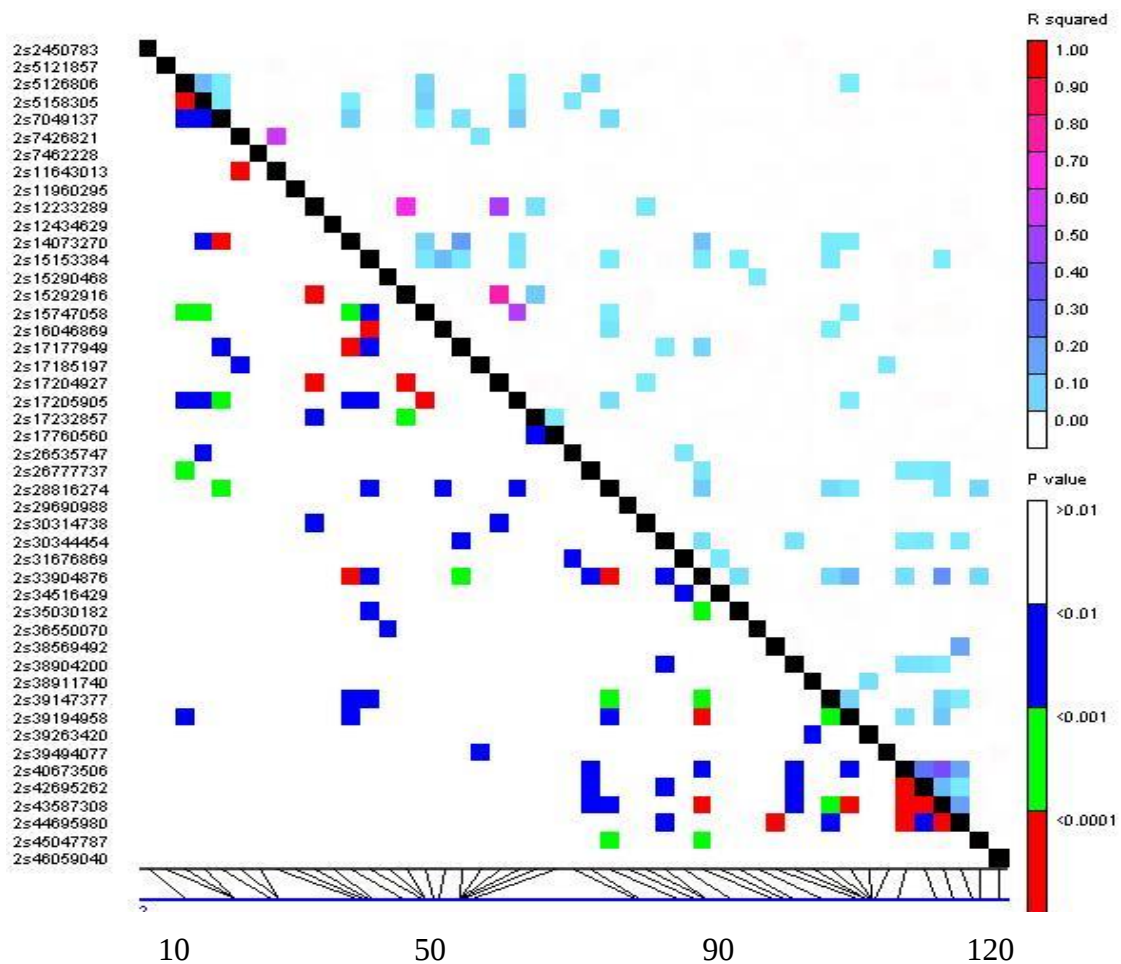
2016, 2017 & BLUE: 1. Stamen length, 2. Branching Pattern, 3. Dry Matter Content, 4. Flower Color, 5. Growth Habit, 6. Pistil Length, 7. Leaf Shape, 8. Maturity, 9. Number of leaflets, 10. Number of Tubers per Plant, 11. Plant Height, 12. Specific Gravity, 13. Pistil length above stamen, 14. Stem Thickness, 15. Tuber Flesh Color, 16. Tuber Shape, 17. Tuber Skin Color, 18. Tuber Skin Texture, 19. Tuber Size, 20. Tuber Yield

4.4 Linkage Disequilibrium (LD)

Linkage Disequilibrium (LD) is the non-random association between two different loci, and is a pairwise measurement between the polymorphic sites. The steadfastness and influence of association mapping studies in a bunch of cultivars rely on the degree of LD that depends on the size of sample, mating system, history of population, frequency of recombination, mutation and chromosome region over the entire genome (Ersoz *et al.*, 2009; Chao *et al.*, 2010; Zhang *et al.*, 2009). Linkage Disequilibrium decay is an outcome of genetic distance. LD can decay in a short as well as in long distance constructed on the population and the species under study and the region of the chromosome under study. Association mapping achieve the events of historical recombination since LD is the consequence of all the recombination events that has arose since the origin of an allele through mutation. Only loci that are attached strictly persist to be connected and co-segregate for various generations (Morton *et al.*, 2001). This offers the prospect to separate the traits that are quantitative in nature with mapping of very high resolution at gene level (Ersoz *et al.*, 2009); So, contributing genes having even diffident effects can be mapped by using LD-based association tactics (Hirschorn and Daly, 2005).

Some spontaneously accessible specific computer software are available, for example “GOLD” (graphical overview of linkage disequilibrium) [Abecasis *et al.*, 2000], for the representation of population structure and LD patterns, “TASSEL” (Trait Analysis by aSSociation, Evolution and Linkage) (Whitt *et al.*, 2003; <http://www.maizegenetics.net/tassel/>) and Power Marker (Liu *et al.*, 2005) also contain such kind of graphical display features. In association mapping, the sturdy LD block constructions are of a prodigious importance, which streamlines LD mapping exertions of multifaceted traits (Zhang *et al.*, 2002). In association mapping, linkage disequilibrium blocks are beneficial when it comes to the sizes. Which recommend the requirements for the minimum number of markers to professionally cover the whole genome haplotype blocks in association mapping. To get graphical display of pairwise LD as well as LD decay plot, TASSEL software was used. Graphical presentation of pairwise linkage disequilibrium between two different loci is very beneficial for the evaluation of LD patterns measured using many molecular markers. Pairwise Linkage Disequilibrium can be embodied as a triangle plot (Figure 4.1) built on important pairwise Linkage Disequilibrium level (r^2 , and p -value as well as D') that aids to envisage the loci block (red blocks) in valuable LD.

Pairwise LD values of polymorphic sites were plotted on both the X- and Y-axis; At the upper side of the diagonal, r^2 values are displayed and below that diagonal the diagonal, corresponding p -values are displayed from rapid 1000 shuffle permutation test. Every cell characterizes the assessment of two sets of marker sites with the color codes indicating the occurrence of substantial LD. For the significance threshold level, colored bar code is shown. The gage for genetic distance was manually drawn for a hypothetical genome fragment.

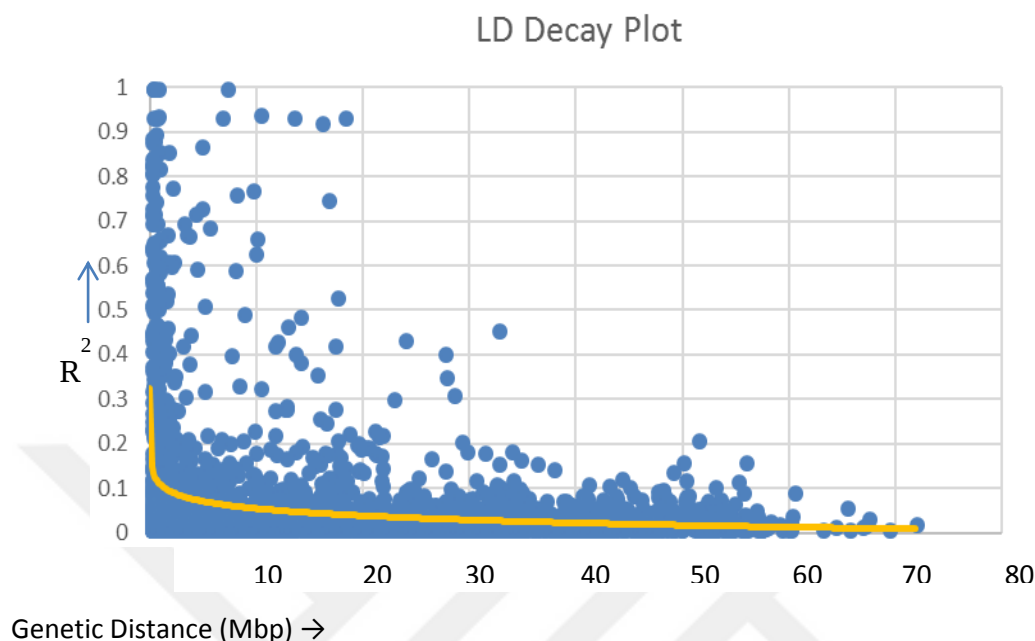


A supposed genome fragment (Base pairs in Mbs)

Figure 4.4. The triangle plot for pairwise Linkage Disequilibrium between SNP marker sites in a genome fragment of 2nd chromosome of potato

A pairwise linkage disequilibrium values (r^2) are designed against a genetic distance. Internal trend line is a nonlinear logarithmic regression curve of r^2 on genetic distance. The threshold for LD decay is considered below $r^2 = 0.1$ and based on this trend line it

was found to be around 1.98 Mb in above plot. A pairwise LD between unlinked marker loci is assigned to 80 Mbs distance point.



LD decay= 1.98 Mb, LD Decay is considered below $R^2 = 0.1$ threshold

Figure 4.5. LD decay plot portrayed from LD values of a proposed marker data to demonstrate a mean size of genome-wide LD block

To know the sizes of these LD blocks, r^2 values (otherwise, D') usually plotted against the weighted (bp) or genetic (cM) distance stated as a “LD decay plot” (Figure 4.2). One can investigate a mean LD decay genome-wide plotting LD values attained from a data set covering an entire genome (i.e., with evenly spaced markers across all chromosomes in a genome) against distance. Instead, the degree of LD for region (gene or chromosome) can be predictable from an LD decay plot generated using dataset obtained from a region of interest. When such a LD decay plot is generated, usually, distance point is looked for, where LD value (r^2) starts decreasing below 0.1 or half of D' strength ($D' = 0.5$) based on curve of nonlinear logarithmic trend line (Whitt et al., 2003; Remington et al., 2001; Hamblin et al., 2004). This gives the rough estimates of the extent of LD for association study, but for the estimates to be more precise, highly significant threshold LD values ($r^2 \geq 0.2$) are also used as a limit. The decline of the LD within the genetic distance specifies that the part of LD is preserved with linkage and is relative to the recombination (Gupta et al., 2005; Stich et al., 2005). Linkage disequilibrium values are given chromosome wise in Table 4.1.

Table 4.2. Linkage disequilibrium in the genotypes used

Chr.	Number of Markers	Mean Distance (Mbs)	r^2	Significant LD (%)	LD Decay (Mb)
1	735	15.75	0.016	48.4	1.64
2	237	15.70	0.013	47.2	1.84
3	365	19.03	0.100	49.1	1.80
4	197	25.85	0.040	49.4	1.91
5	246	17.09	0.030	50.4	1.99
6	235	19.32	0.170	49.1	2.29
7	189	17.79	0.130	49.0	1.95
8	200	19.48	0.140	49.5	2.15
9	227	24.81	0.100	48.2	2.14
10	329	7.00	0.140	49.8	1.85
11	321	17.22	0.160	49.9	2.24
12	304	23.04	0.170	49.7	1.99
Linked Markers	3585	18.50	0.101	49.1	1.98

We observed that the nonlinear trend line of the LD measure r^2 vs. the physical map distance decayed with almost 1.98Mbs (2.475 cM) to an r^2 value of 0.10 (Figure 4.2). In an AFLP marker dataset used in 221 tetraploid potato genotypes, r^2 value dropped below 0.1 at about 3 cM (D'hoop et al., 2008). This result was considered as promising for LD mapping in potato and its applications in marker assisted breeding by the same authors. Simko *et al.*, (2006) found that the r^2 value reached to 0.21 in around 1 kb distance and to the value of 0.10 at the distance of around 10 cM, which shows a considerable slow LD decay as per our study. D'hoop *et al.*, (2010) observed that LD decayed on average at about 5 cM to r^2 values of 0.1. These results were obtained from the study containing 3300 AFLP markers and around 650 microsatellite alleles. Based on the genome size of potato that is of 844 Mbp (Xu *et al.*, 2011) and a length of the genetic map in the order of 1000 cM (Gebhardt et al., 1991), 5 cM resembles to closely 4 Mbp. This evaluation of LD decay in case of magnitude is almost the same that we observed in our study and stipulates the incidence of large haplotype blocks in the population analyzed by D'hoop et al., (2010).

4.5. Association Mapping

4.5.1 Plant Traits

4.5.1.1 Growth Habit (GH):

Growth habit of the plants was analyzed and was found with normal distribution curve over the range of readings that were taken. For validating either the model that we are practicing is appropriate for controlling population stratification, family structure as well as the false positives among the samples, quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale were drawn and following results were obtained.

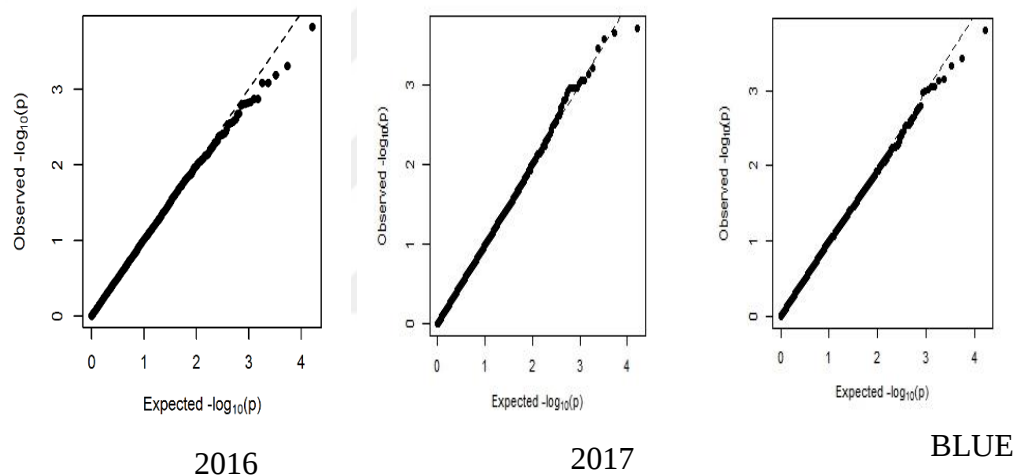


Figure 4.6. Q-Q plot for growth habit of potato for the years 2016, 2017 and BLUE

We observed through the results that up to the reading of 2.5 the graph was as smooth as the expected Q-Q plot, but after 2.5 to 4 the observed value graph was not per expected line in case of growth habit of the potato plants. All the plots from each data set was found quite normal in which expected lines were being followed by the observed p values coming from our values. There were some points at the end of each year graph which showed some deviation from expected values but still it is thought to be normal. Generalized linear model was used to analyze the data through the statistical software TASSEL 4.0.13 standalone version and following Manhattan plots were formulated based on three data set that is 2016, 2017 and BLUEs.

In 2016, there were different markers that were above the significant line and showing some association with growth habit (Table 4.3).

Table 4.3. SNP found in 2016 data set from GLM for growth habit

SNP	Chromosome	Putative functions	Base pair	P Value
solcap_snp_c2_16530	1	Peptidyl-prolyl cis-trans isomerase	6401	8.00E-07
solcap_snp_c2_25435	12	Conserved gene of unknown function	2603	1.28E-06
solcap_snp_c2_31193	6	Conserved gene of unknown function	473	3.99E-06

In 2016 data, on chromosome 1, there is a SNP named as solcap_snp_c2_16530 is associated with growth habit of potato. On Chromosome 12, solcap_snp_c2_25435 and on Chromosome 6, solcap_snp_c2_31193 is associated with the growth habit of potato plants.

For the data of year 2017, there were some significant markers that were associated with growth habit (Table 4.4).

Table 4.4. SNP found in 2017 data set from GLM for growth habit

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_16530	1	Peptidyl-prolyl cis-trans isomerase	6401	1.39E-06
solcap_snp_c1_2150	11	Ring finger protein	1358	1.88E-06
solcap_snp_c2_16470	1	Gene of unknown function	6399	3.68E-06
solcap_snp_c2_25692	3	N-acetyl-glutamate synthase	2612	4.59E-06

In 2017 data, solcap_snp_c2_16530, solcap_snp_c2_16470 were associated with growth habit on chromosome 1, solcap_snp_c1_2150 on chromosome 11 and solcap_snp_c2_25692 on chromosome 3 were associated with the growth habit of potato plants.

For the values of BLUEs, there were some significant markers (Table 4.5).

Table 4.5. SNP found in BLUE data set from GLM for growth habit

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_16530	1	Peptidyl-prolyl cis-trans isomerase	6401	1.37E-06
solcap_snp_c2_52789	1	Uncharacterized protein	8343	2.30E-06
solcap_snp_c2_25435	12	Conserved gene of unknown function	2603	2.76E-06
solcap_snp_c2_16470	1	Gene of unknown function	6399	3.05E-06

In the data set of BLUE, 3 SNPs namely solcap_snp_c2_16530, solcap_snp_c2_52789 and solcap_snp_c2_16470 on 1st and one SNP, solcap_snp_c2_25435 on 12th chromosome were associated with growth habit.

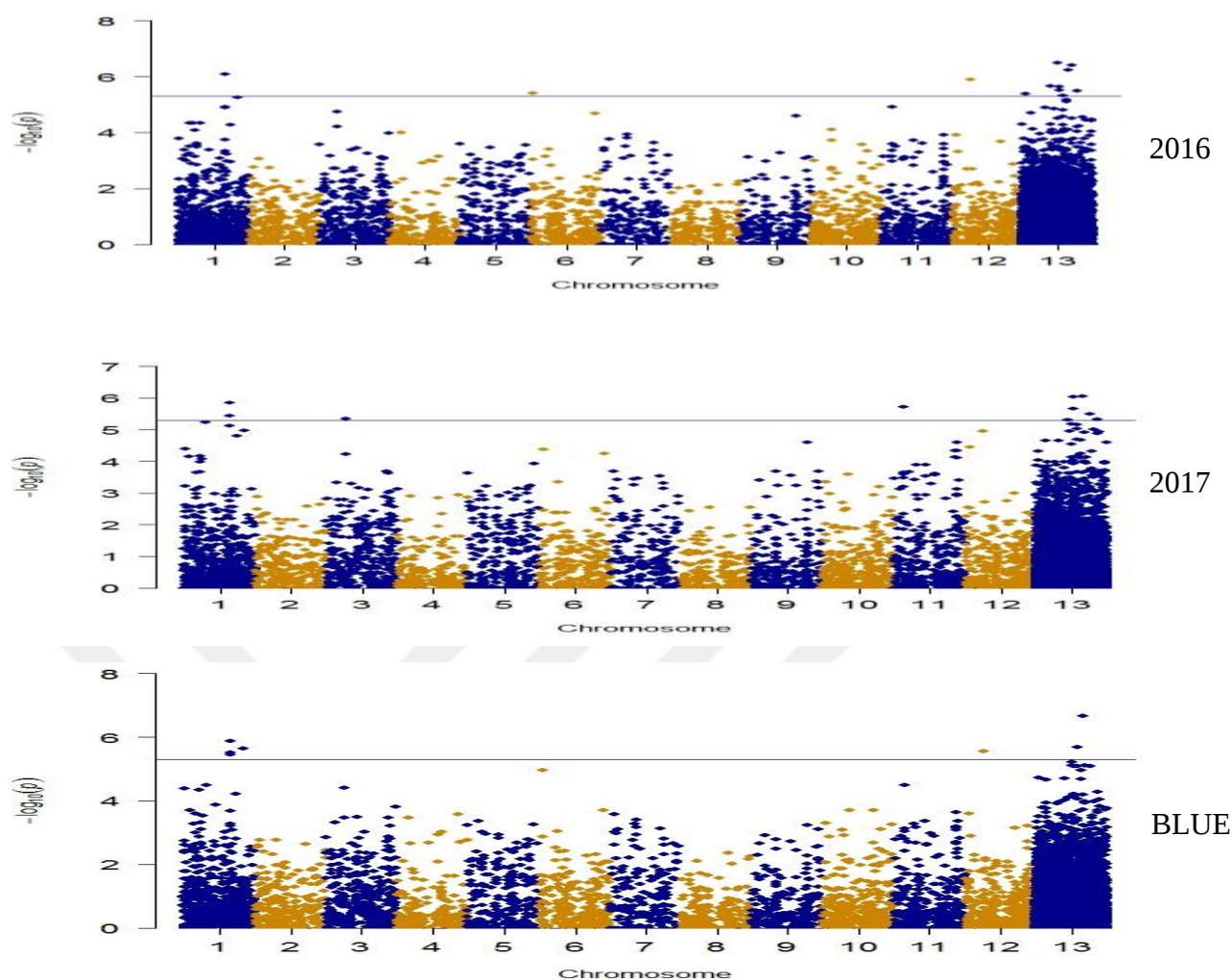


Figure 4.7. Manhattan plots from generalized linear model for growth habit of potato plants. Chromosome #13 are the unmapped markers

It was observed that SNP (solcap_snp_c2_16530) was found to be associated in all three data sets i.e. 2016, 2017 as well as for BLUEs. solcap_snp_c2_25435 was found to be commonly associated in 2016 and BLUEs. solcap_snp_c2_16470 was found to be commonly associated among the data sets of 2017 and BLUEs.

When Mixed linear model was used to analyze the data through the statistical software TASSEL 4.0.13 standalone version and following Manhattan plots were formulated based on three data sets (2016, 2017 and BLUEs). The results from MLM showed that the SNPs associated with growth habit of potato was linked on chromosome 1 and chromosome 12 (Table 4.6).

Table 4.6. SNP found in 2016 data set from MLM for growth habit

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_25435	12	Conserved gene of unknown function	1572457	1.89E-06
solcap_snp_c2_16530	1	Peptidyl-prolyl cis-trans isomerase	75928651	2.98E-06

For the data of year 2017 and BLUEs there was no SNP marker associated with growth habit using MLM package.

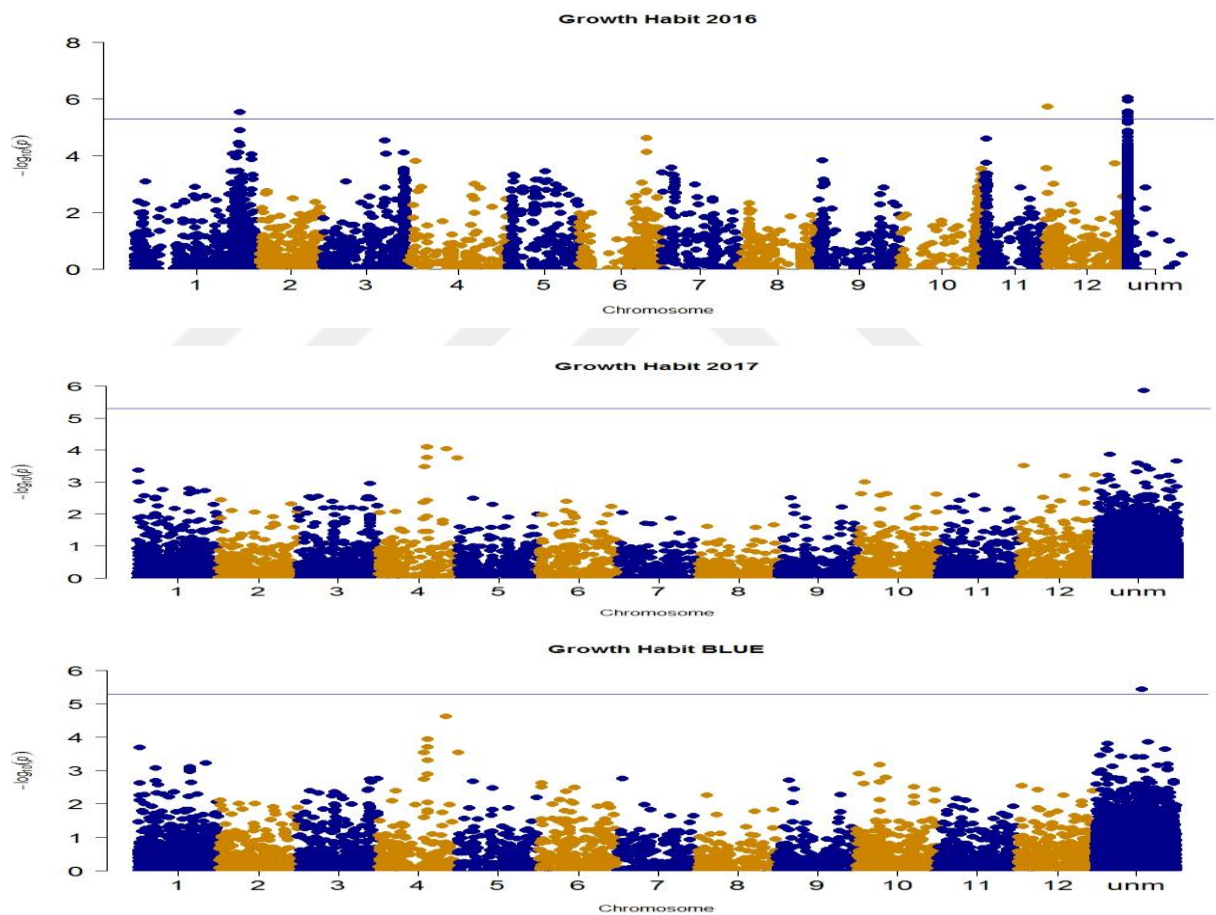


Figure 4.8. Manhattan plot through mixed linear model for the year 2016, 2017 and BLUE

Johanna et al., 2015 worked on growth patterns in *Arabidopsis* and remained unsuccessful in finding the association between SNPs specifically for this trait. Larson et al., 2006 explained that two of the four fringe QTLs were perceived in homoeologous regions of linkage groups 3a and 3b in both families, indicating that one gene may control much of the dramatic difference in growth habit between *Leymus cinereus*

(Scribn. & Merr.) A. Love and *L. triticoides* (Buckley) Pilg. are tall caespitose and short rhizomatous perennial Triticeae grasses. Devos and Gale, 2000 explained that chromosome number 3 is preserved for the trait of growth habit in Triticeae. Babb and Muehlbauer, 2003; Franckowiak, 1996 explained that the genes that alter the trait of growth habit in Barley has been localized on the chromosome 3HL of Barley. Lawrence et al., 2004 described growth habit maize and explained that it is associated on the chromosome 4. Ware et al., 2002 found growth habit in rice to be linked on the chromosome number 11. In our studies in both GLM and MLM we found the association for this trait on chromosome 1, 3, 5, 6, 11 and as well as on 12. In our study we found solcap_snp_c2_16530 on chromosome 1 to be associated with growth habit trait in both the models and for 2016, 2017 as well as for BLUE. So, this marker can be used for crop breeding programs in future.

4.5.1.2 Branching Pattern (BP)

Branching Pattern of the plants was analyzed and was found with normal distribution curve over the range of readings that were taken. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale were drawn and following results were obtained. The curve was found to be bending down at the end showing the observed p values less than the expected p values while reaching at the end this shows that our model that we are implementing is good enough to control population stratifications. Still this graph is said to be a normal curve showing pretty similar readings as per expected.

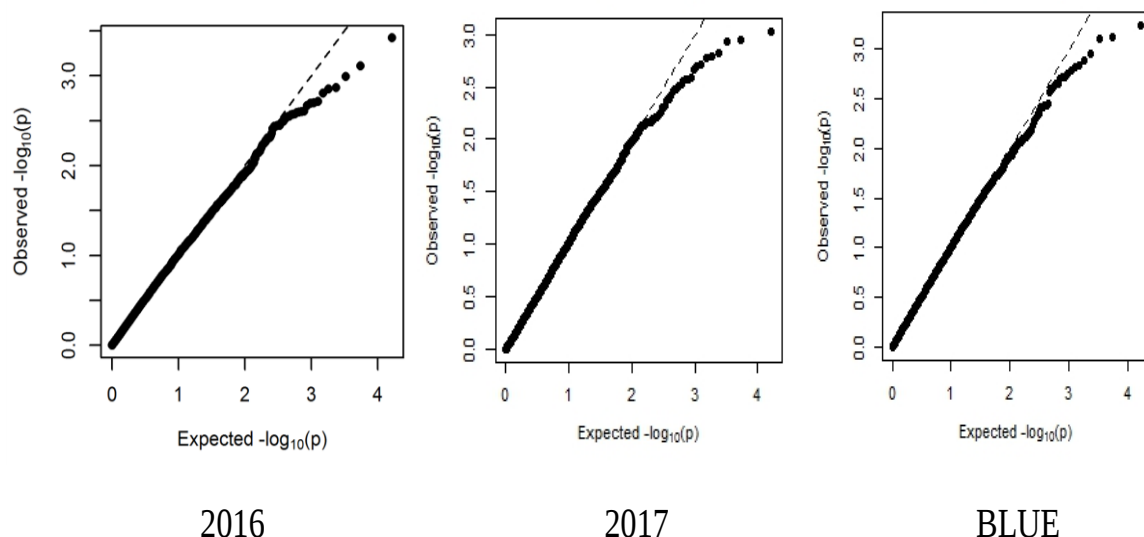


Figure 4.9. Q-Q plot for the trait branching pattern of potato crop for the year 2016, 2017 and BLUE

From the results of Q-Q plots we observed that the fair part of Q-Q plot of observed values followed the model's expected line while at the end the observed value seemed to be laying down of the expected line showing that the model that we applied is fairly stringent to control the false positives and population stratification. Generalized linear model was used to analyze the data through the statistical software TASSEL 4.0.13 standalone version and following Manhattan plots were formulated based on three data set that is 2016, 2017 and BLUEs.

In data of 2016, six SNPs were associated with the branching pattern and those were located on chromosome 1, 3 and some of them were unmapped (Table 4.7).

Table 4.7. SNP found in 2016 data set from GLM for branching pattern

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c1_3874	1	Conserved gene of unknown function	1464	1.14E-06
solcap_snp_c2_9611	3	Auxin-responsive protein IAA17	8870	2.40E-06

Table 4.7. (Continue) SNP found in 2016 data set from GLM for branching pattern

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_9557	3	N-alpha-acetyltransferase 15, NatA auxiliary subunit	4618	3.13E-06
solcap_snp_c1_131	3	probable sulfate transporter 3.4	1081	3.94E-06

While in 2017 data and in Best Linear Unbiased Estimator values there was no SNP found to be associated with branching pattern trait of the plants. Manhattan plots for each three data sets are as follows.

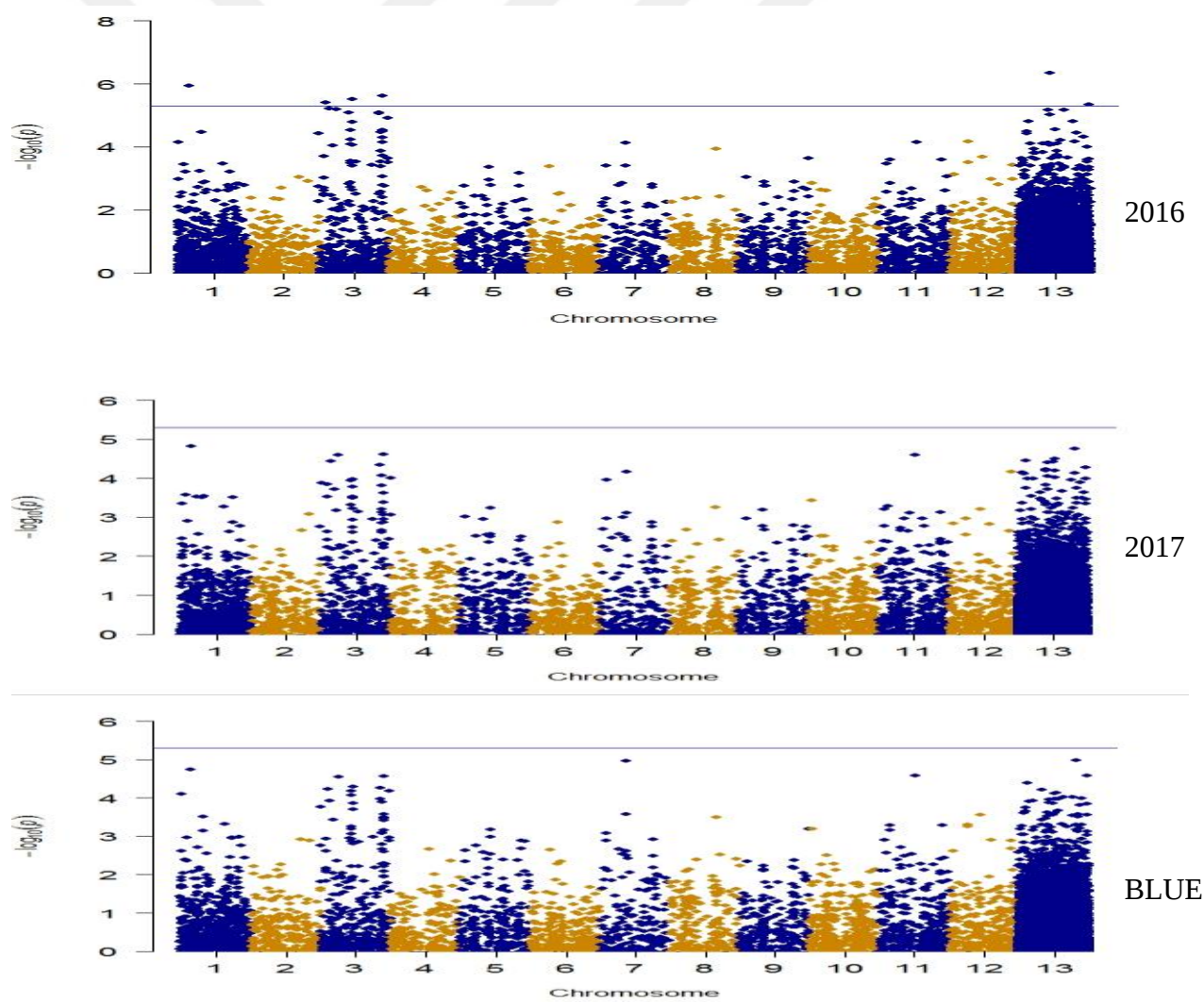


Figure 4.10. Manhattan plots from generalized linear model for branching pattern

Mixed linear model was also applied for the results. We got the following results in case of MLM,

There was no significant associated marker for all three data sets in case of branching pattern when we used ML model.

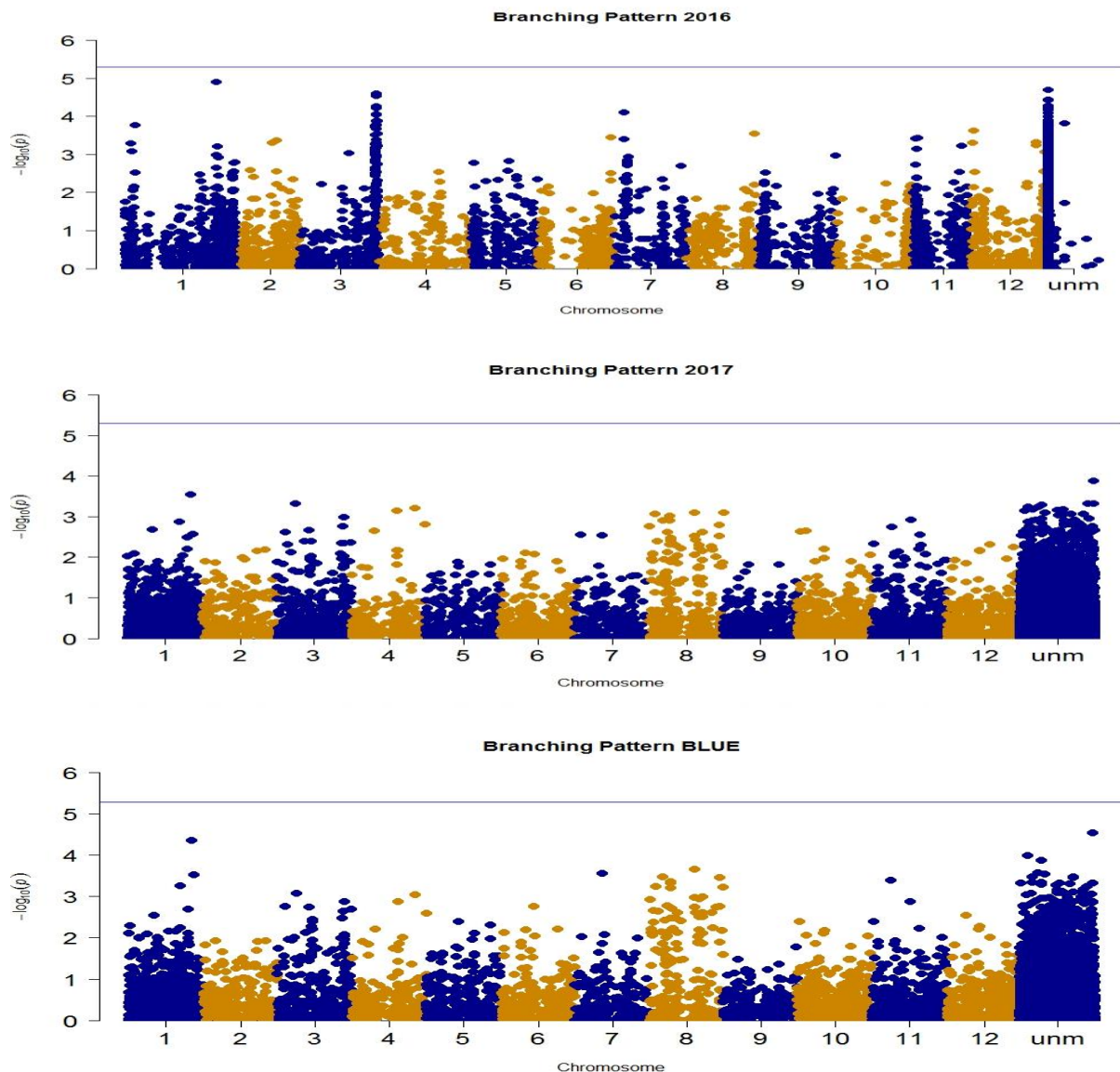


Figure 4.11. Manhattan plots from mixed linear model for the trait branching pattern

Nambeesan et al., 2015 while doing association mapping in sunflower considering apical and basal branches revealed that branching habit in sunflower is being controlled on 10th chromosome as sunflower totally have 17 chromosomes. In case of our findings it was found that branching habit is associated on chromosome number 1 and 3. So, for the next breeding programs we can use SNPs on chromosome 3 (solcap_snp_c2_9611, solcap_snp_c2_9557 and solcap_snp_c1_131)

4.5.1.3 Plant Height (PH)

Plant Height of the plants was analyzed and normal distribution curve was found over the range of readings that were taken while flowering. For validation, either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected $-\log_{10} p$ values versus observed P values at $-\log_{10}$ scale and following results were obtained. The curve was found to be ending down at the end showing the observed p values less than the expected p values. This means that in the data set while performing bar plots analysis there are some curves that are not appropriate and have some tails at the end or may be some abnormal distribution is there. But still these types of Q-Q plots are said to be quite normal.

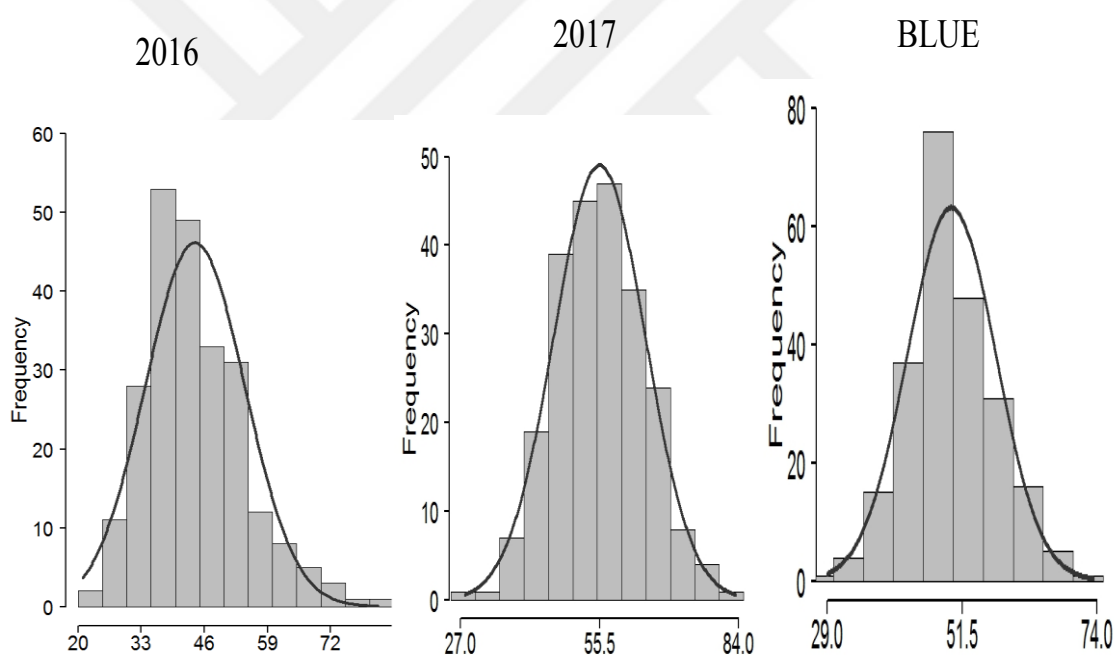


Figure 4.12. Bar plots for the year 2016, 2017 and BLUE for plant height

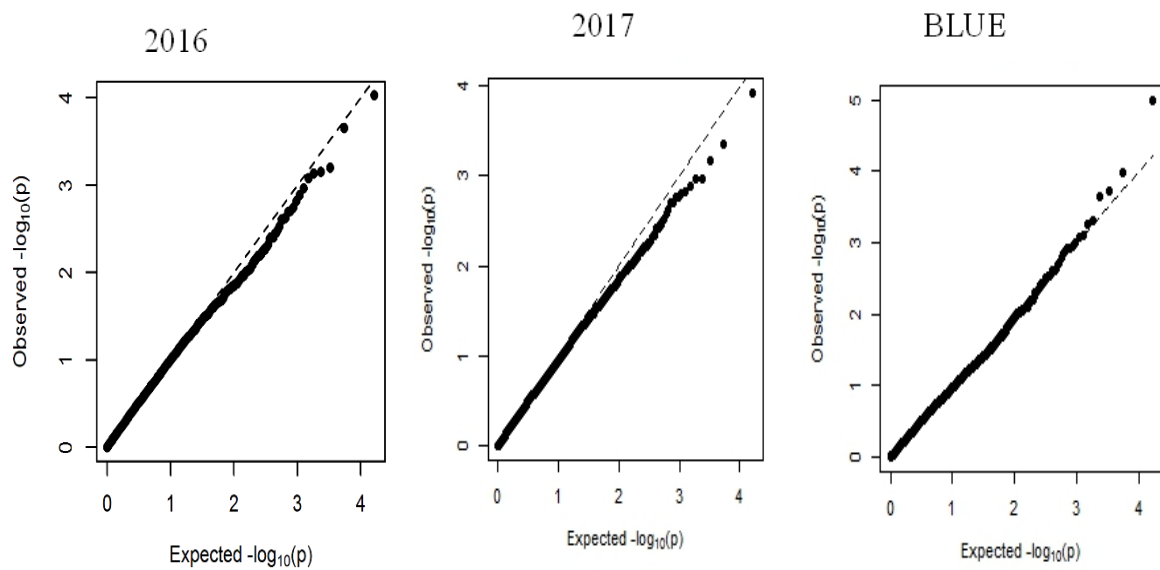


Figure 4.13. Q-Q plot for plant height for potato crop for the year 2016, 2017 and BLUE

From the results of Q-Q plots we came to know that the observed values came from our experiments followed fairly the expected values line keeping the line similar. For the year 2016 and 2017 the observed line remained below the expected line but for the data set of BLUE it stood different as four points at the end was found above from the expected values giving some clue of associations among the SNPs

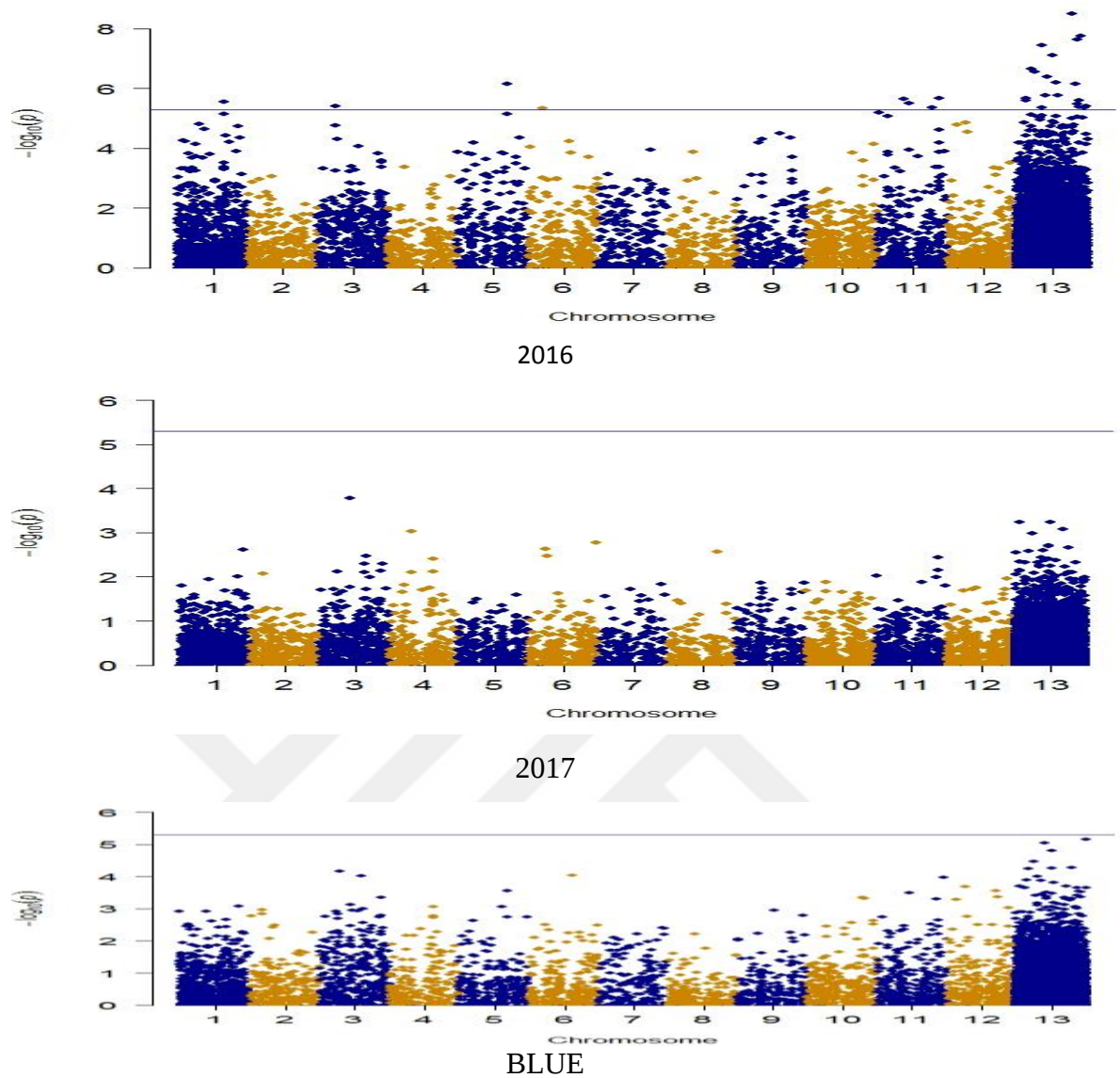


Figure 4.14. Manhattan plots as per GLM for the trait plant height of potato crop for the year 2016, 2017 and BLUE

In the data of 2016, there were 08 markers that were associated with plant height. Some of them were associated with chromosome at 1, 3, 5, 6 and 11th chromosome (Table 4.8).

Table 4.8. SNP found in 2016 data set from GLM for plant height

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_24373	5	Two-component response regulator ARR11, transcript variant X1	6804	7.13E-07
solcap_snp_c2_55972	11	Uncharacterized	8527	2.15E-06
solcap_snp_c2_47386	11	Uncharacterized	3744	2.24E-06
solcap_snp_c2_16530	1	DNA-directed RNA polymerase V subunit 5A	6401	2.79E-06
solcap_snp_c2_57886	11	Uncharacterized	4384	3.15E-06
solcap_snp_c2_25692	3	Pentatricopeptide repeat-containing protein At3g49240	2612	3.96E-06
solcap_snp_c2_39960	11	Clone RH195J04	7590	4.24E-06
solcap_snp_c2_16823	6	Uncharacterized	2201	4.66E-06

While in data for the data set of 2017 and BLUE there was no SNP marker that was found to be associated with plant height of the potato plants.

After GLM, same trait was analyzed with Mixed Linear Model and following results were obtained.

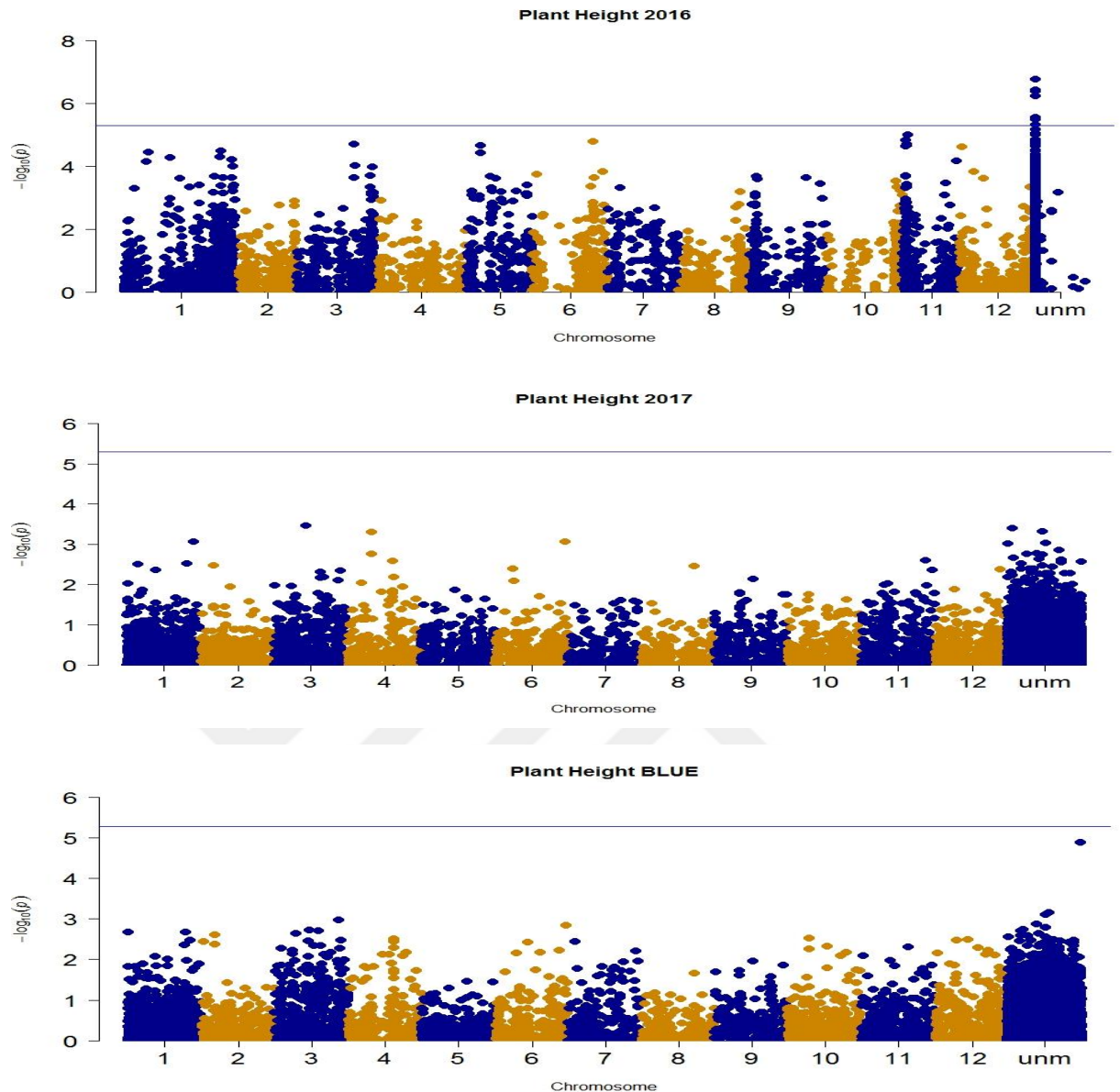


Figure 4.15. Manhattan plots as per MLM for plant height trait of potato crop

No significant SNP marker was found in any data set i.e. 2016, 2017 and BLUEs.

Fei-Fei et al., 2016 detected some significant QTLs while studying agronomic and quantitative traits in rice using SSR marker system. They found that plant height trait is associated with chromosomes 6 and 7 of rice. In our studies we also found the related results that plant height trait was associated with chromosome 1, 3, 5, 6 and on chromosome 11. As 04 SNP markers out of 08 are linked to chromosome number 11 of potato in our studies so we could use these markers as candidate markers for our future breeding programs after being validated.

4.5.1.4 Stem Thickness (ST)

Stem thickness of 10 plants were analyzed and normal distribution curve was found over the range of readings that were taken while flowering. For validation, either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected $-\log_{10}$ p values versus observed P values at $-\log_{10}$ scale and following results were obtained. The curve was found to be bending down at the end showing the observed p values less than the expected p values. This means that in the data set while performing bar plots analysis there are some curves that are not appropriate and have some tails at the end or may be some abnormal distribution is there. But still these type of Q-Q plots are said to be quite normal and data can be trusted for further association analysis.

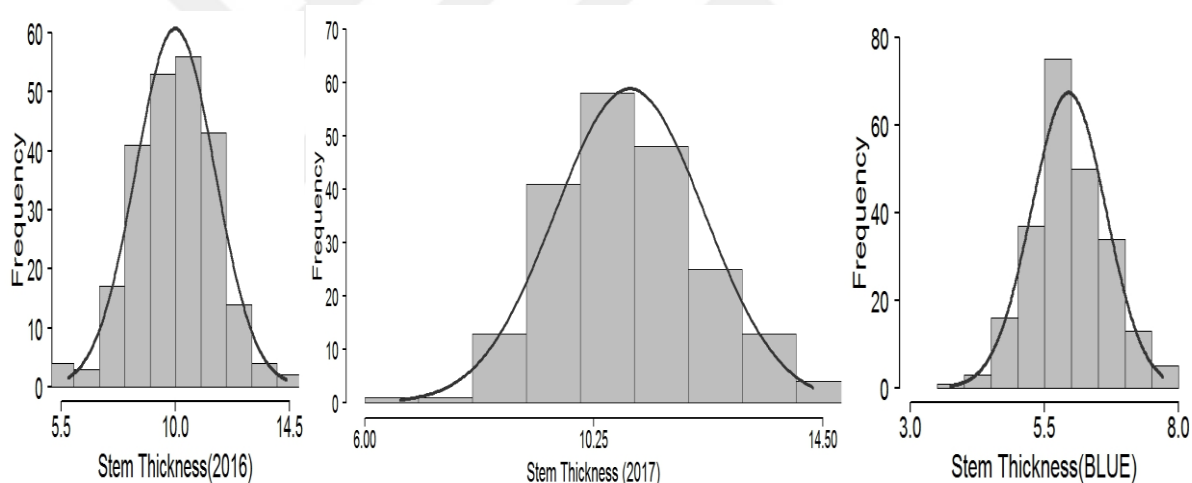


Figure 4.16. Bar plots for stem thickness traits for the year 2016, 2017 and BLUE.

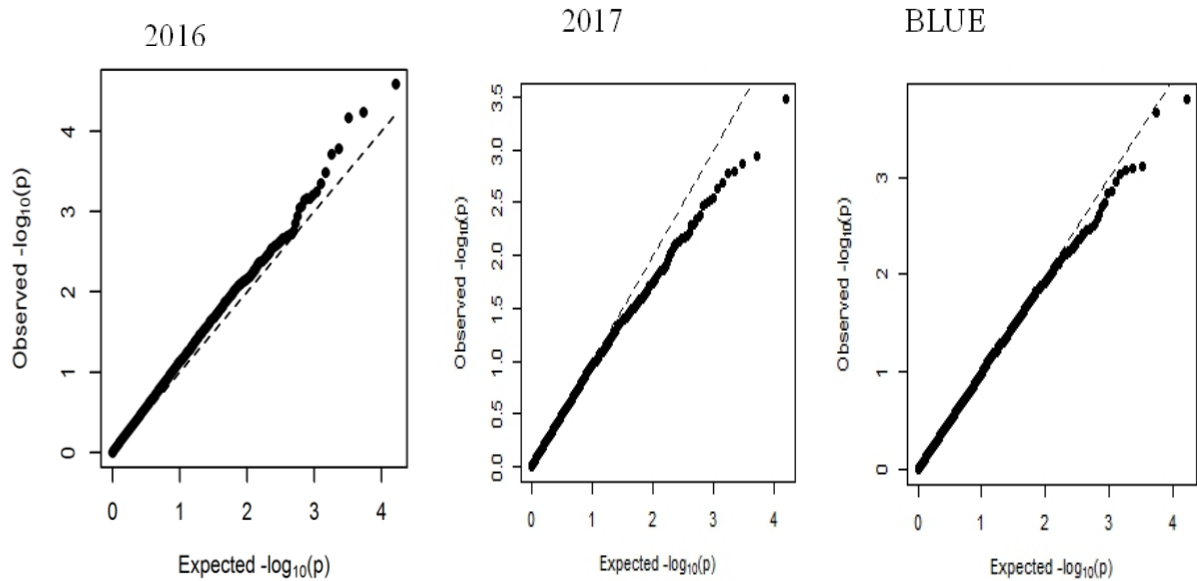


Figure 4.17. Q-Q plots for stem thickness for the year 2016, 2017 and BLUE

After getting Q-Q plots we observed that in the data set of 2016 the observed values followed the expected values up to 2.5 but after that there was some deviation and the observed values went above the expected values. For the data set of year 2017, up to the point of 1.5 the readings of observed and expected values were similar but after that the observed values got lighter and went below the expected line and shown very slow growth and even at the end these were found to be far below from the expected line from the model. While in the case of data set of BLUE, we observed the uniform movement of both expected as well as the observed values but at the end there were two to three points that were found below the expected line.

Taking about the results in Manhattan plots there was also not similar results just like Q-Q plots. In the data of year 2016 and 2017 we couldn't find any significant markers associated with the stem thickness trait of the crop. The reason of not finding any SNP could be environment genotype interaction. It could be the conditions that affected its overall linkage to the specific traits. While in BLUEs we found a couple of SNPs that were associated with stem thickness. One of the associated SNP markers remained unmapped and the remaining one was on chromosome number 11. SNP located on chromosome 11 has the following features (Table 4.9).

Table 4.9. SNP found in BLUE data set from GLM for stem thickness

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_4274	11	Auxin-induced beta-glucosidase	7698	4.28E-06

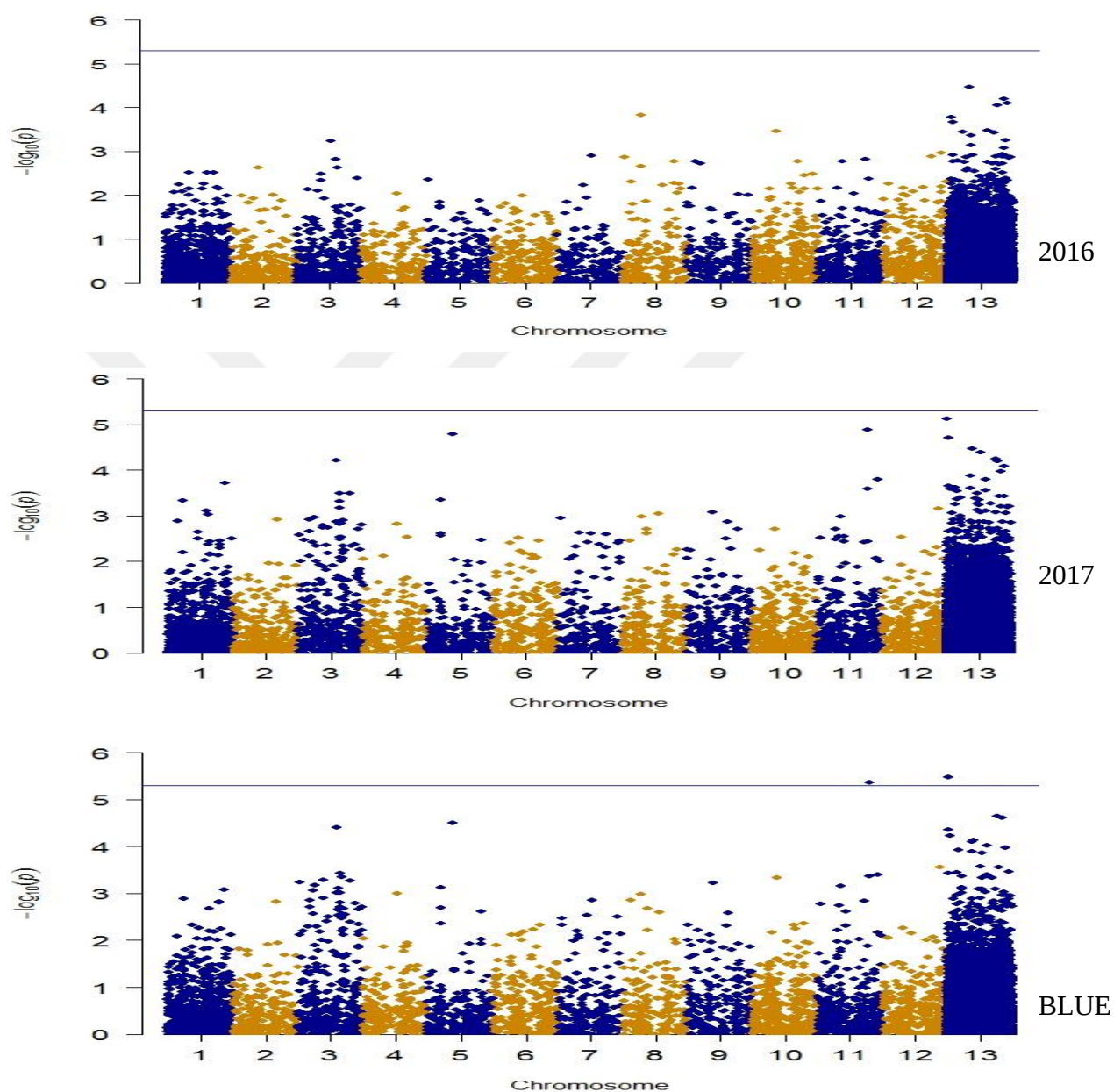


Figure 4.18. Manhattan plots for stem thickness using GLM

After having analyzed with GLM, we performed MLM for our data set and found the following results,

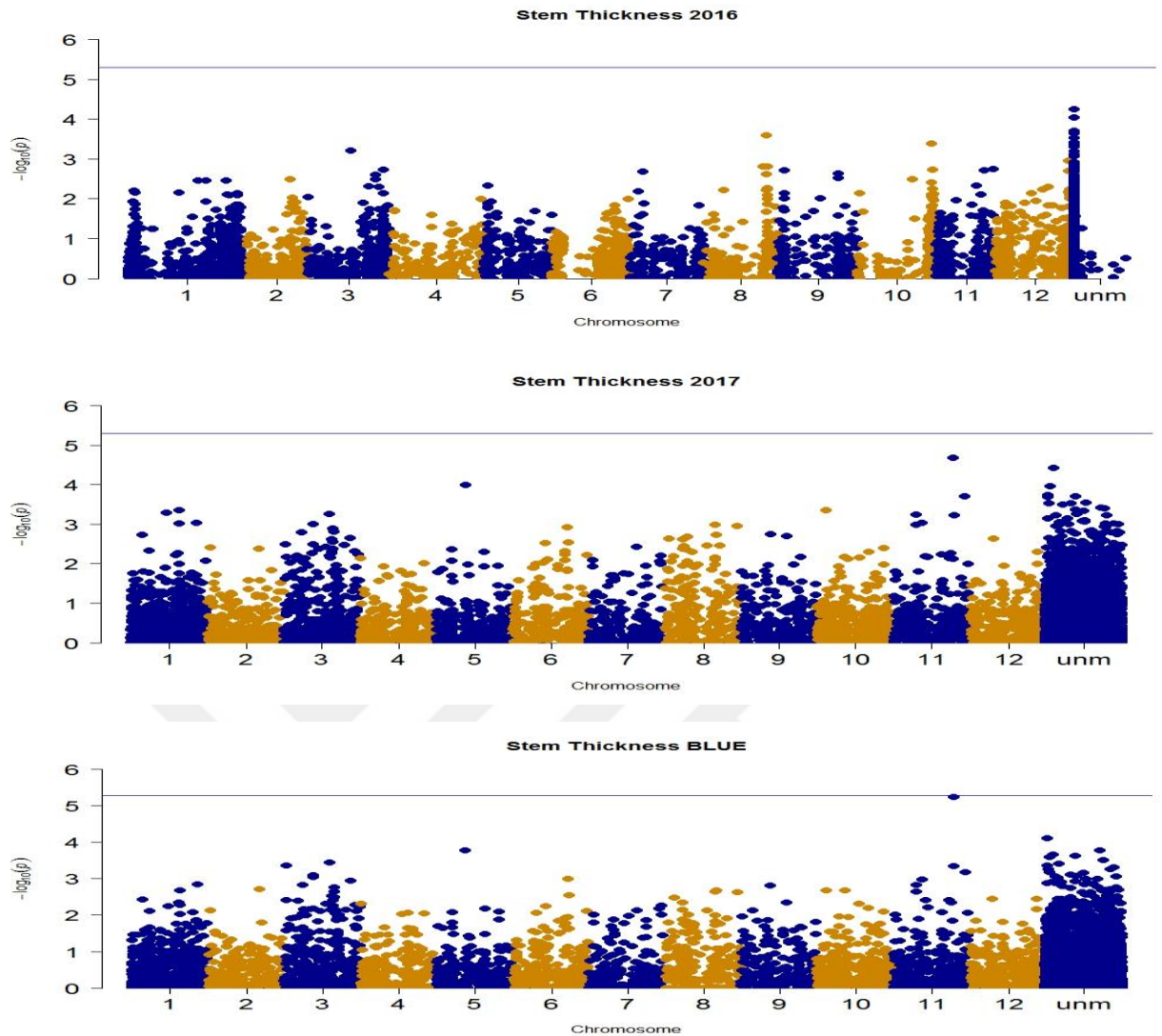


Figure 4.19. Manhattan plots for the trait stem thickness using MLM

No significant marker was found to be associated with stem thickness using mixed linear model. Just in BLUEs on chromosome 11 there was a single SNP very near to the threshold level, but it was not associated with the traits.

Several studies have identified QTLs linked to stem diameter in wheat (Berry and Berry, 2015, Hai et al., 2005, Keller et al., 1999), and in rice Kashiwagi et al., 2008 four stem diameter QTLs (sdm1, sdm7, sdm8, sdm12; NIL28) were found. In our studies we found an SNP linked on chromosome 11 of potato genome.

The gene found in GLM on chromosome 11 is found to be associated with different functions like biological and molecular functions. In molecular functions it is involved in Catalysis of the hydrolysis of any O-glycosyl bond. While in case of biological

functions it is involved in the chemical reactions and pathways involving carbohydrates, any of a group of organic compounds based of the general formula $C_x(H_2O)_y$. Includes the formation of carbohydrate derivatives by the addition of a carbohydrate residue to another molecule. So, the genes like this can be useful for further studies for carbohydrate metabolic studies in crop species. This SNP should be validated before future breeding studies in MAS as well as in GS.

4.5.1.5 Leaves Shape (LS)

Leaves shape of the plants were observed phenotypically and then it was analyzed with genotypic data for the association of significant markers with the definite trait. Throughout the reading a uniform curve was observed showing that this trait is a quantitative trait. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected $-\log_{10} p$ values versus observed P values at $-\log_{10}$ scale and following results were obtained. The curve was found to be almost accurate in years 2016 data as following the normal curve and slide rise at the end of the graph. In year 2017, this curve was found to be below then the marked line showing that the observed values are less than the expected p values. While for BLUEs the readings were in between 2016 and 2017 data.

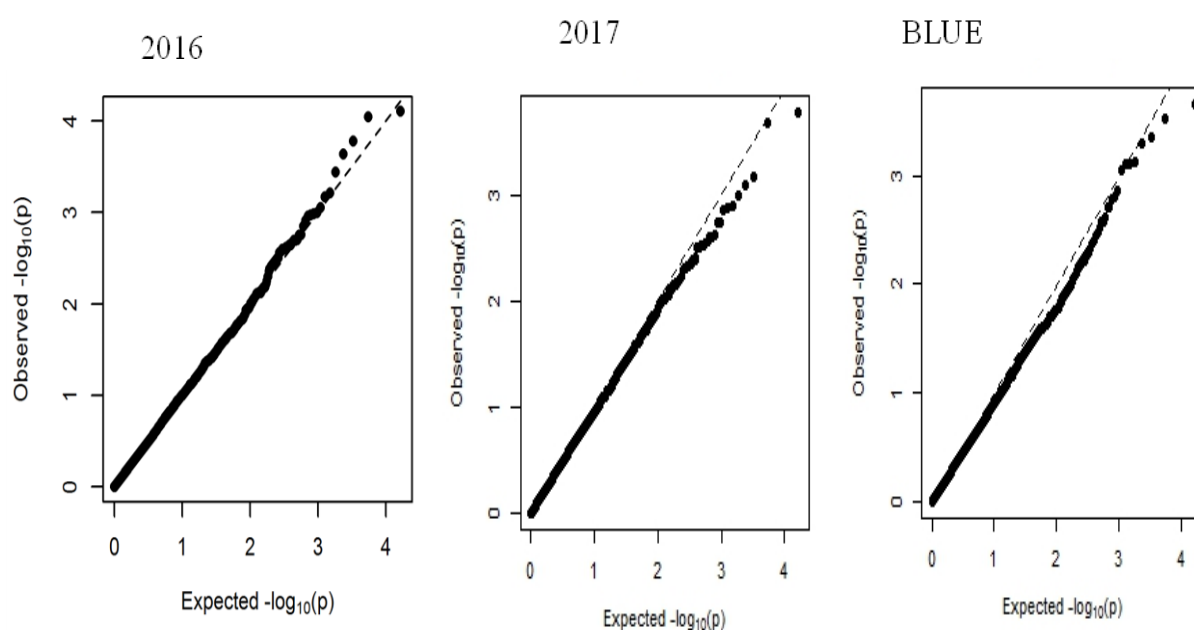


Figure 4.20 Q-Q plot for leaves shapes trait of potato

While going through the Manhattan plots were observed the followings results.

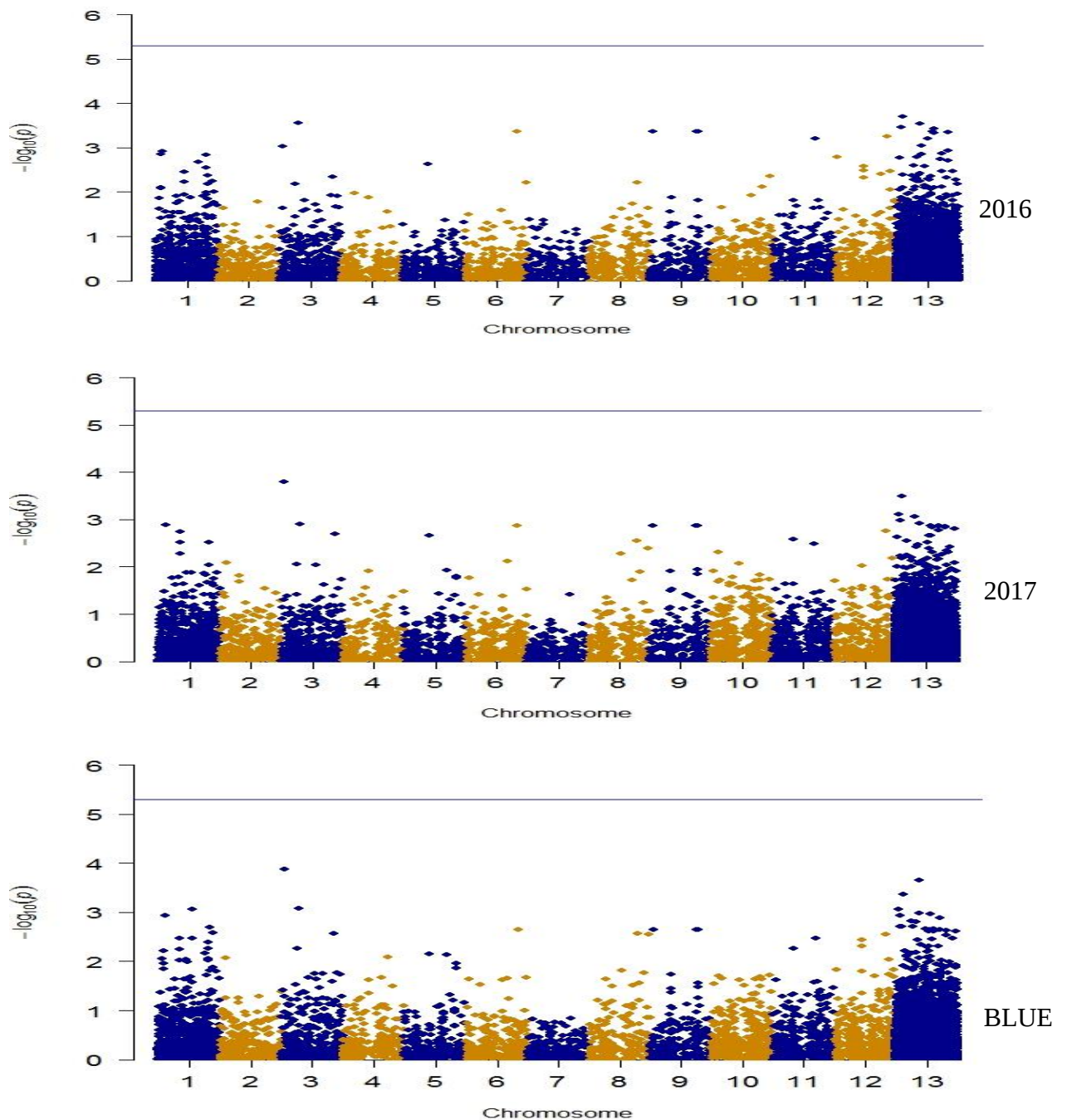


Figure 4.21. Manhattan plots for leaves shapes using GLM

In all the three data, the results were similar with the Manhattan plots having no significant SNP marker associated with the trait of Leaf Shape. All the SNPs remained below the threshold level that was marked at 5% of probability of finding false positives in our samples.

After being analyzed with GLM, all data sets were analyzed again with MLM but no significant marker was found in any data set for leaves shape.

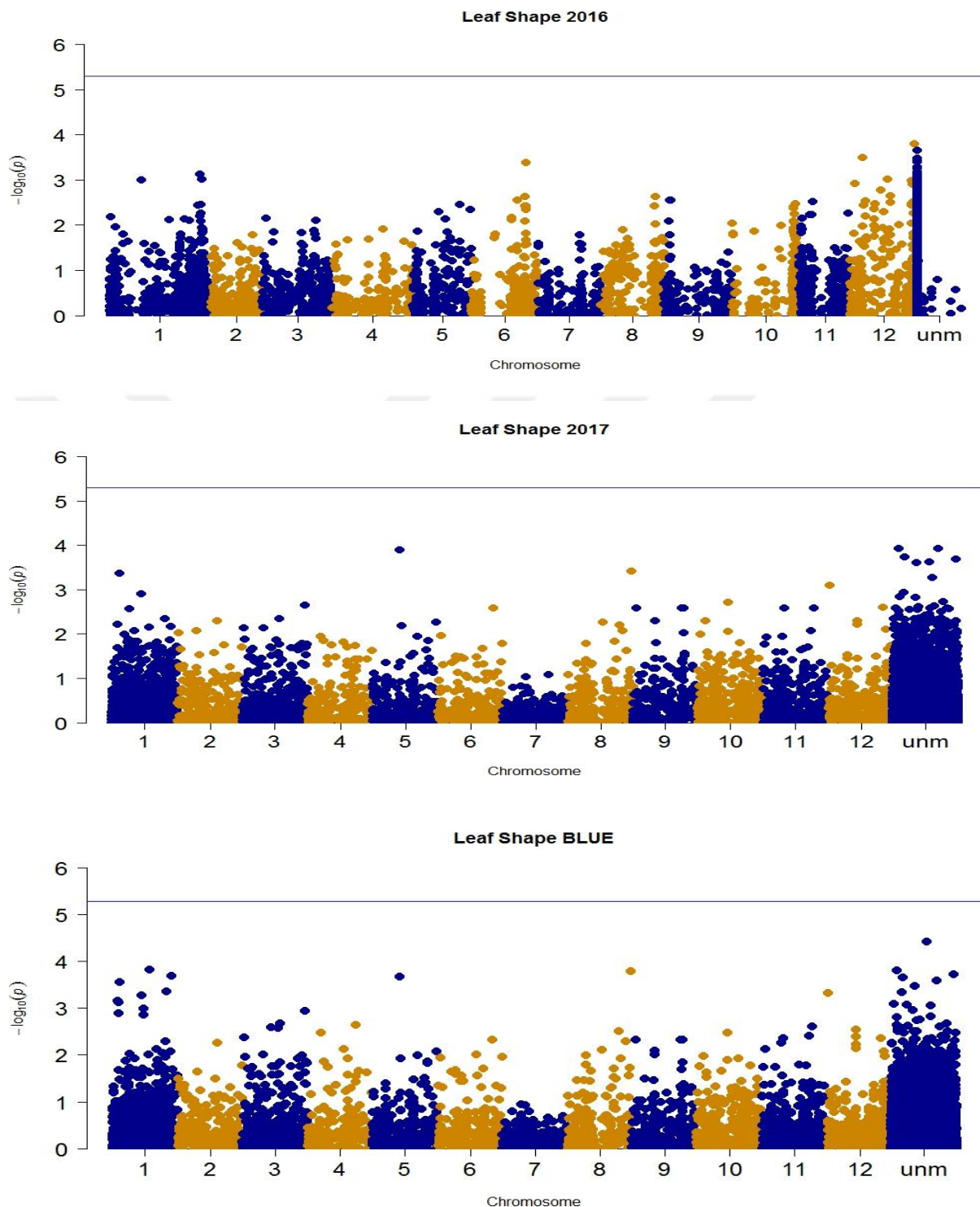


Figure 4.22. Manhattan plots for leaves shapes using MLM

There is not any study that discuss particularly leaf shape trait in potatoes as well as in other crops. However, there are some papers with leaf morphology in different crops covering different characteristics. Jian et al., 2017 explained in their study on maize that

leaf morphology trait is located on 12 chromosomes of maize with one major QTL located on chromosome A01.

4.5.1.6 Number of Leaflets per Plant (NL)

Number of leaflets of the plants was observed phenotypically and then it was analyzed with genotypic data for the association of significant markers with the definite trait. Uniform curve was observed showing that this trait is a quantitative trait. For validation of the model that we are implementing is stringent for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected $-\log_{10}$ p values versus observed P values at $-\log_{10}$ scale and following results were obtained. Q-Q plot for the year 2016 the plot was similar with the expected p values line. In data of year 2017 the curve was deviating from the expected p values having more values as per expected and thus the line was also at the higher place. In BLUES the p values of observed and the expected p values was similar thus the both lines stood side by side showing the normal flow. If the line in Q-Q plot is deviating in the graph, this means that in the data set while performing bar plots analysis there are some curves that are not ideal that could have some tails at the end or may be some abnormal distribution is there. We can see in each data set that the distribution curve is not ideal and have some flaws that's why q-q plot is deviating from expected. But still these type of Q-Q plots are said to be quite normal and data can be trusted for further association analysis.

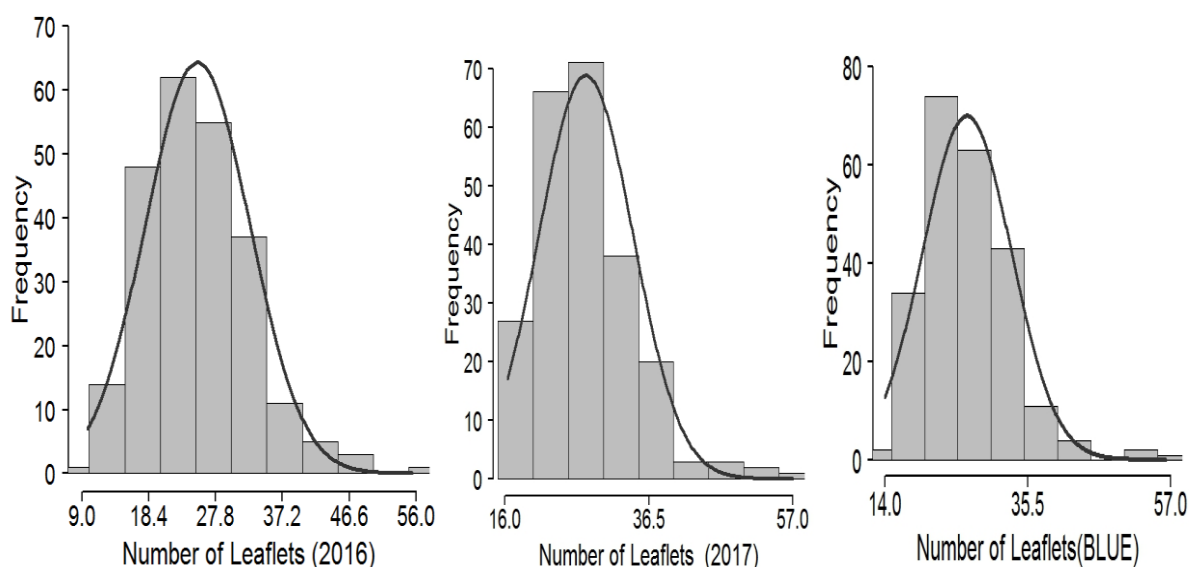


Figure 4.23. Bar plots for number of leaflets per plant

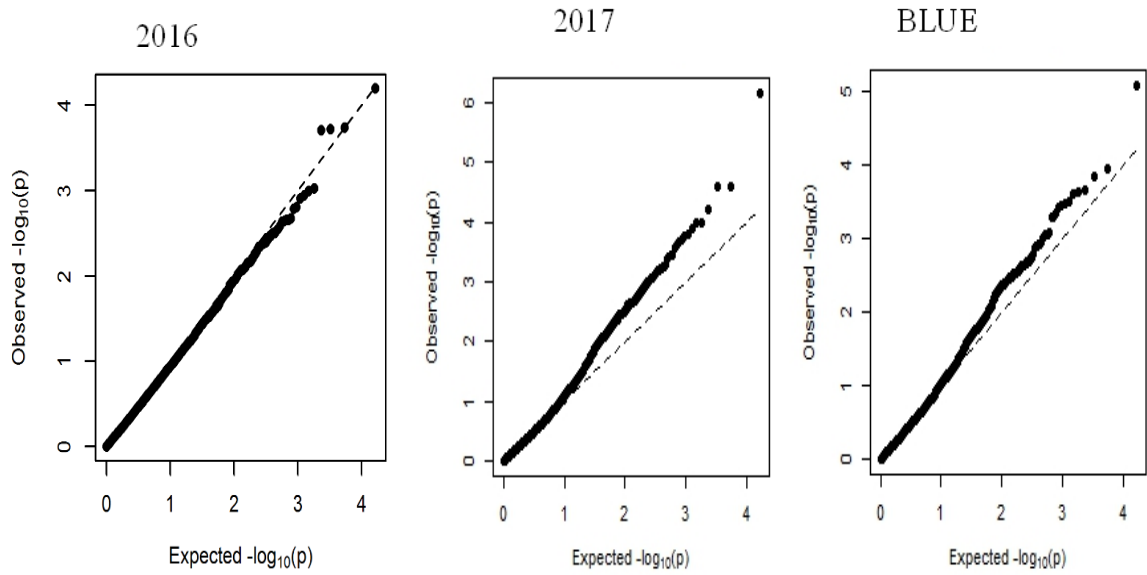


Figure 4.24. Q-Q plots for number of leaflets per plant for the year 2016, 2017 and BLUE

In Manhattan plots and finding some significant markers associated with number of leaflets per plant, we plotted Manhattan plots using p values that we got from TASEEL software. We found no significant SNP that is associated with number of leaflets per plants for each data i.e. 2016, 2017 and BLUEs.

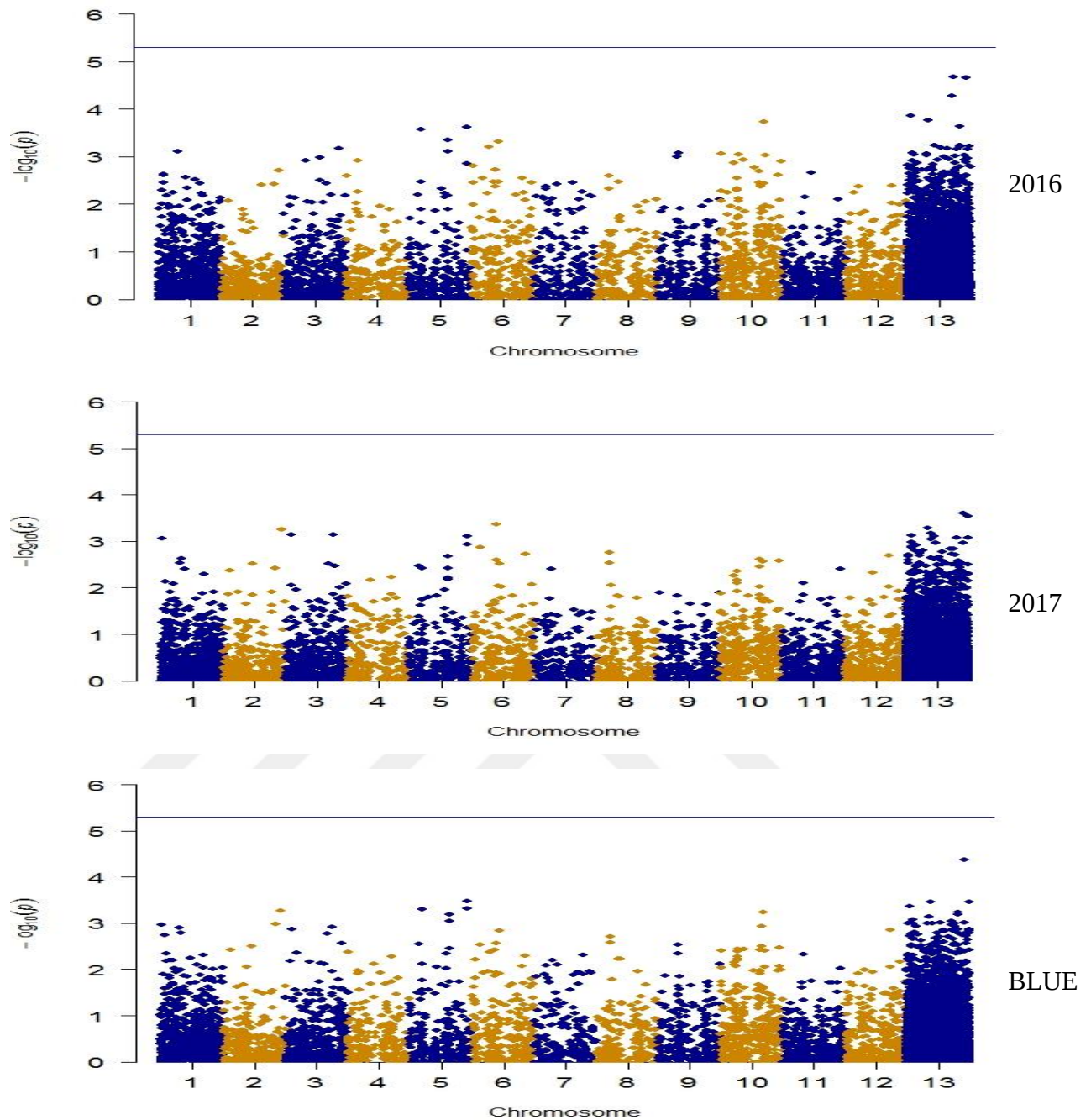


Figure 4.25. Manhattan plots obtained from GLM for the trait number of leaflets per plant

After being analyzed with GLM, all data sets were analyzed again with MLM, but no significant marker was found in any data set for number of leaflets.

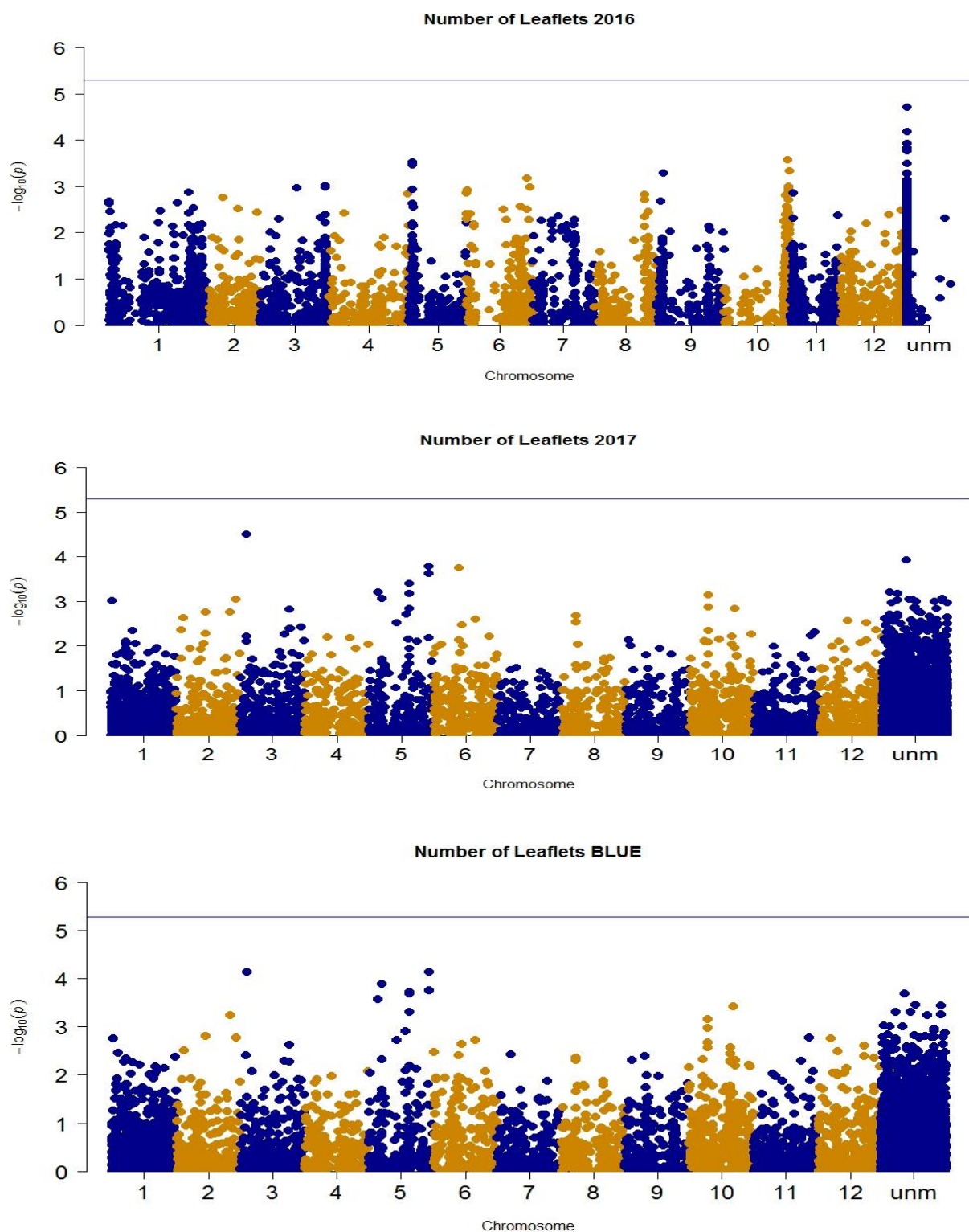


Figure 4.26. Manhattan plots obtained from GLM for the trait number of leaflets per plant

Up to our knowledge there is not such study which describes the trait of number of leaflets per in association mapping. This is the novel trait that we have studied and unfortunately, we were unable to find any association of markers with this trait.

4.5.1.7 Maturity

Maturity date of the plants was observed phenotypically by physical appearance and then it was analyzed with genotypic data for the association of significant markers with the definite trait. Bar plots were plotted to see the uniformity of the curve. Uniform curve was observed showing that this trait is a quantitative trait and covering the range of the values. Yet some difference between the data points was observed which could be due to the environment phenotype interaction that caused the change in phenotypic data. That is why the data points in different year data sets were varied. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected $-\log_{10} p$ values versus observed P values at $-\log_{10}$ scale and following results were obtained. Data from 2016 year showed the ideal Q-Q plot following the same expected p values and at the end showing some significant markers present above the line. Data for the year 2017 also presented the graph following the expected value line and at the end it was also indicating the significant markers that are associated with the traits. While for the data of BLUE, the graph was fine and at the end showing some deviations from the expected values at the very end showing some positive significant markers associated with the trait of interest.

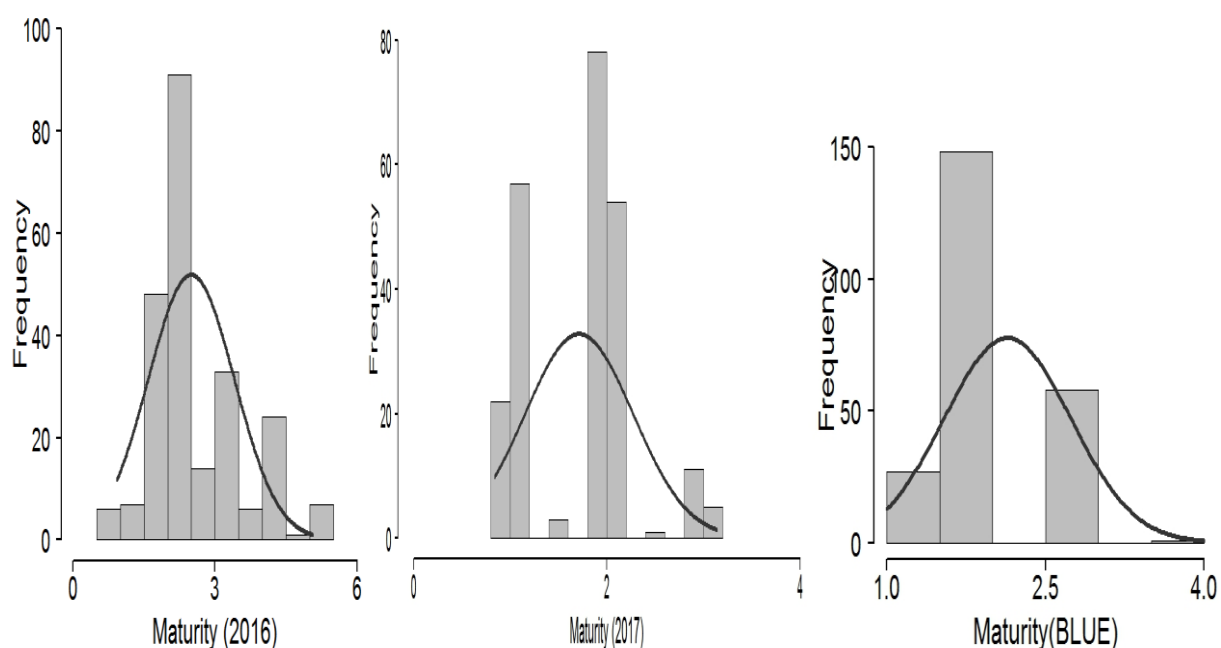


Figure 4.27. Bar plots for maturity of the potato plants

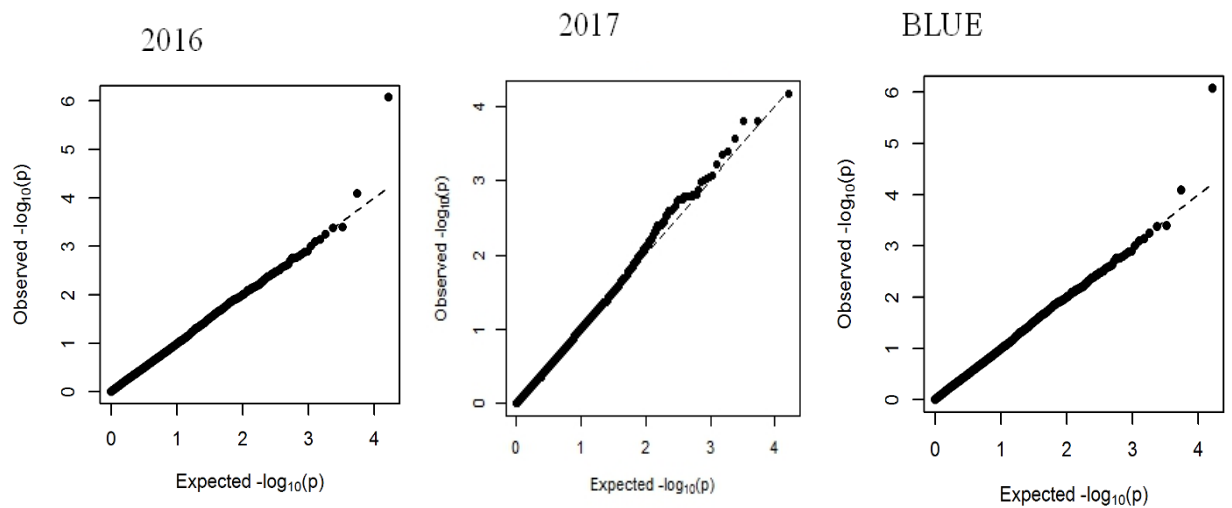


Figure 4.28. Q-Q plots for maturity of the plants for the year 2016, 2017 and BLUE

After having results of Q-Q plots and bar graphs then we planted Manhattan plots to see the actual results and to get the information exactly which marker at which chromosome is significantly attached to the trait of interest and we found the following results.

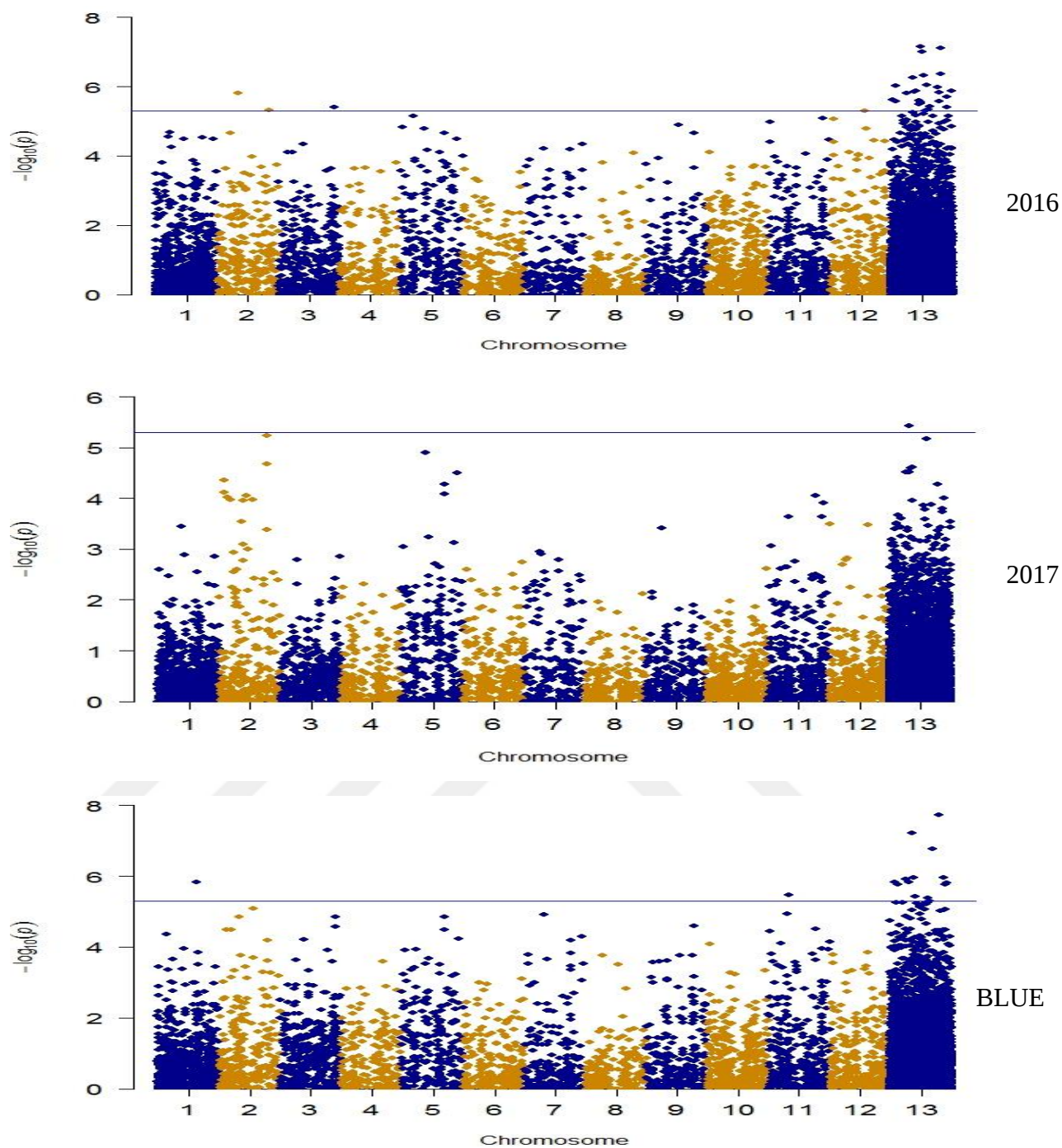


Figure 4.29. Manhattan plots obtained from generalized linear model for maturity

Manhattan plot that we got for the year 2016, we got some SNPs that were significantly associated with maturity of the crops. In those SNPs many markers were among unmapped markers and some of them were on chromosome number 2, 3 and 12 (Table 4.10).

Table 4.10. SNP found in 2016 data set from GLM for maturity

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_40138	2	Sugar carrier protein C	3368	1.56E-06
solcap_snp_c2_9590	3	Uncharacterized	8866	3.92E-06
solcap_snp_c2_51140	2	Proteasome subunit alpha type-5	8251	4.85E-06
solcap_snp_c1_1939	12	Pentatricopeptide repeat- containing protein At3g06430, chloroplastic	5532	5.08E-06

For the data of 2017, only 02 SNP markers were found significant one of them was without any chromosome that mean it was among the unmapped markers and the other SNP was just on the line and it was on chromosome number 2 (Table 4.11).

Table 4.11. SNP found in 2017 data set from GLM for maturity

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_4353	2	4-alpha-glucanotransferase (DE2)	7742	5.75E-06

While for the Manhattan plots coming from BLUEs showed some significant markers that were associated with maturity of the plants and those were with chromosome number 1 and 11 (Table 4.12).

Table 4.12. SNP found in BLUE data set from GLM for maturity

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_14474	1	Nuclear transcription factor Y subunit A-7	6255	1.46E-06
solcap_snp_c2_37164	11	F-box/FBD/LRR- repeat protein At1g13570	3234	3.48E-06

All the associated SNPs in each data set i.e. 2016, 2017 and BLUEs were found to be different from each other. There was no common SNP in all the data sets found to be associated to the trait.

After GLM analysis, we performed MLM analysis for our data set and found a single marker associated except the unmapped markers with maturity of the plants of potato crop in the data set of year 2016. However, there was no associated marker for the data set of 2017 as well as for BLUEs (Table 4.13.)

Table 4.13. SNP found in 2016 data set from MLM for maturity

SNP	Chromosome	Putative Functions	Base pair	P Value
solcap_snp_c2_51140	2	Proteasome subunit alpha type-5	29690874	4.83E-06

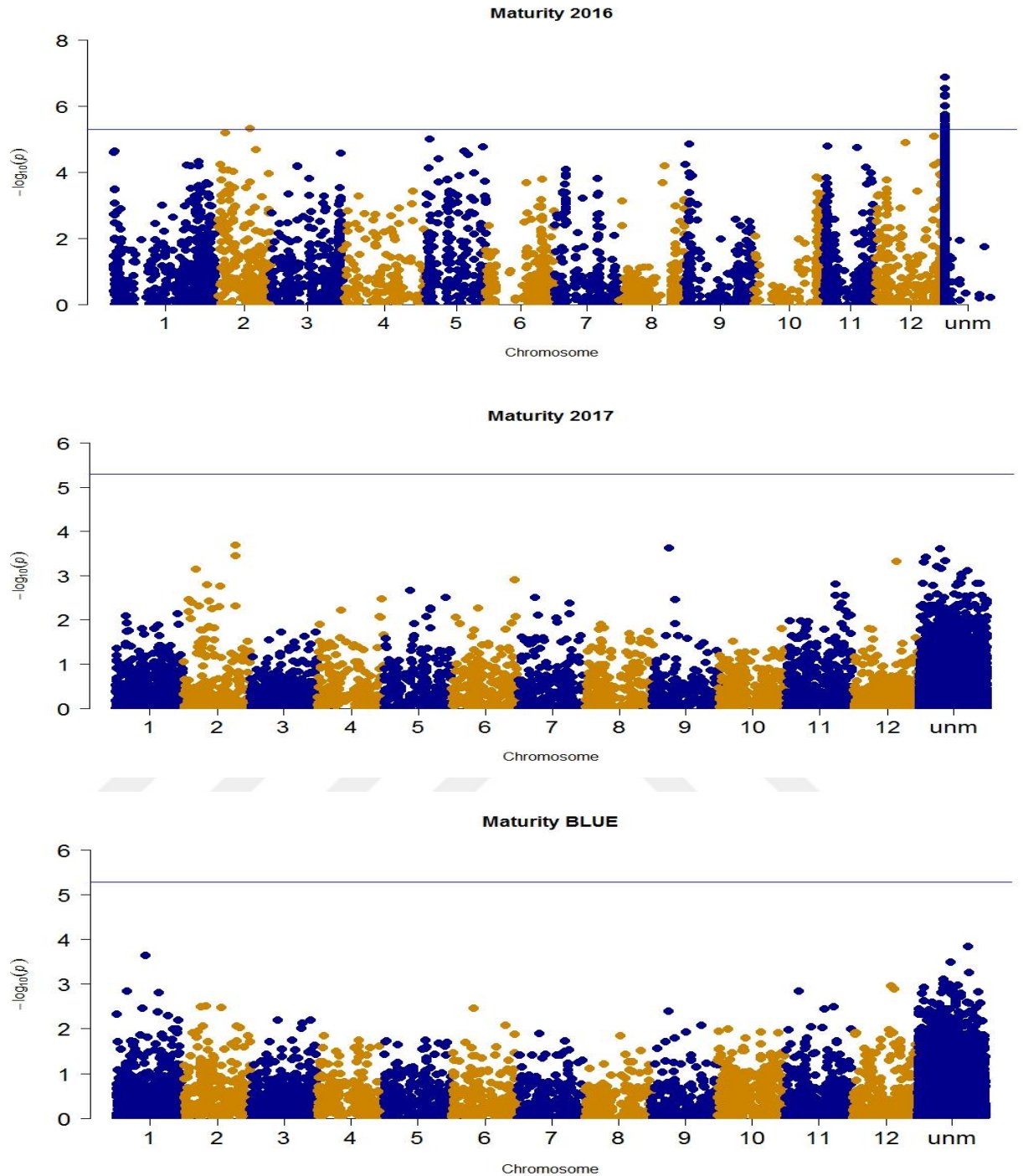


Figure 4.30. Manhattan plots obtained from MLM for maturity of the potato plants

The results from our study in accordance with the findings of Gebhardt et al., 2004 and Visker et al., 2005 who reported that maturity trait is linked on chromosome number 3 and 5. In another study of QTL mapping conducted by Heckett et al., 2014 it was also shown that maturity is associated with the chromosome 5. In our studies we found this trait to be lined on chromosome 1, 2, 3, 11 and 12 complying different model results. For maturity of the potato plants we could consider solcap_snp_c2_51140 marker

present on chromosome 2 for future studies as this marker was found common in both the models.

4.5.2 Flower Traits

4.5.2.1 Flower Color

Flower color of the traits was analyzed manually by their physical appearance and was then analyzed with the genotypic data for the association of significant markers if any with this specific trait. Bar plots were obtained with uniform curve showing the uniformity of data taken for this trait. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Q-Q plots were obtained for flower color showing the uniform line as per expected p values but a bit of deviations at the end that is in the data of year 2016 its going up and suggesting some association of markers with flower color. In the data of year 2017, its bending down a bit but at the end the marker is exactly on the line. For BLUE data the line is same just like for the data of year 2016.

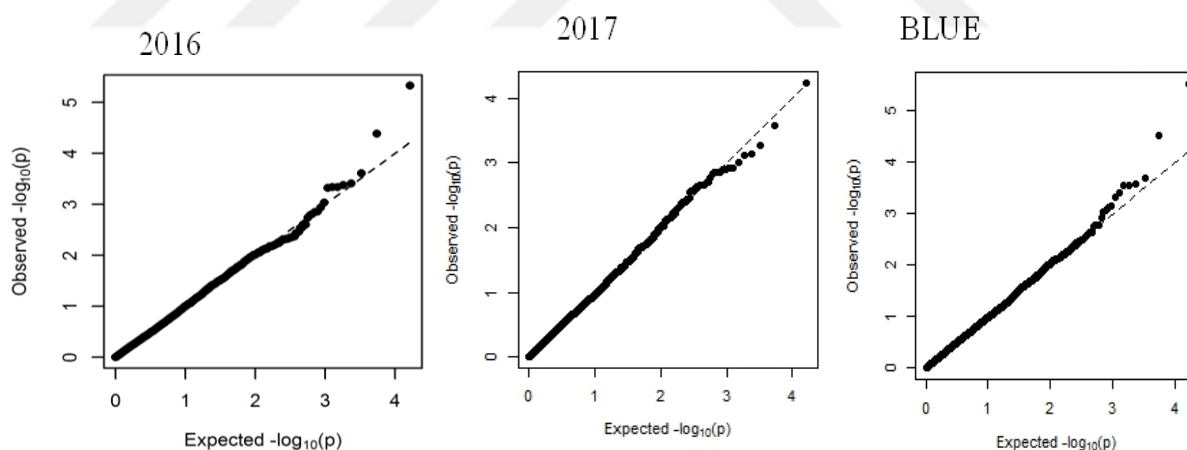


Figure 4.31. Q-Q plots for the year 2016, 2017 and BLUE for the trait of flower color in potato

When Manhattan plots were drawn with the p values that came from TASSEL, we saw that in the data of year 2016, there were two SNPs that were associated with the Flower color. One of them was among the unmapped markers and the remaining one was on the chromosome number 4 (Table 4.14).

Table 4.14. SNP found in 2016 data set from GLM for flower color

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c1_13354	4	Gene of unknown function	5269	5.01E-06

But for the Manhattan plots that were drawn for year 2017 data as well as for BLUEs did not show any significant associated markers. In 2017 data, on chromosome number 1, two markers were found to be very near to threshold line, but they were not above enough to represent the significant associations of those markers to the flower color.

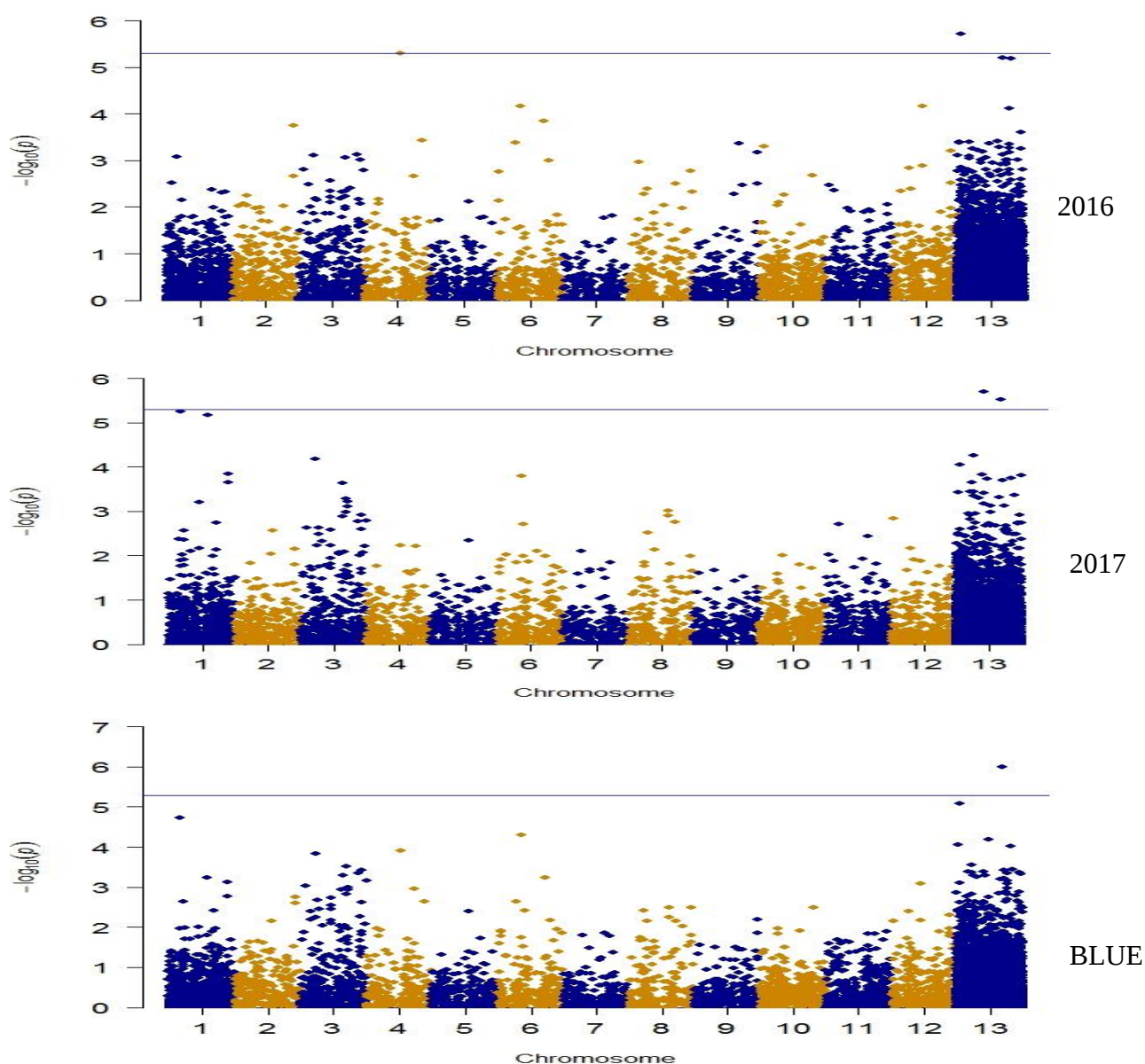


Figure 4.32. Manhattan plots using Generalized Linear Model for Flower color trait in potato

For flower color there was no significantly associated marker when MLM was applied to all data sets.

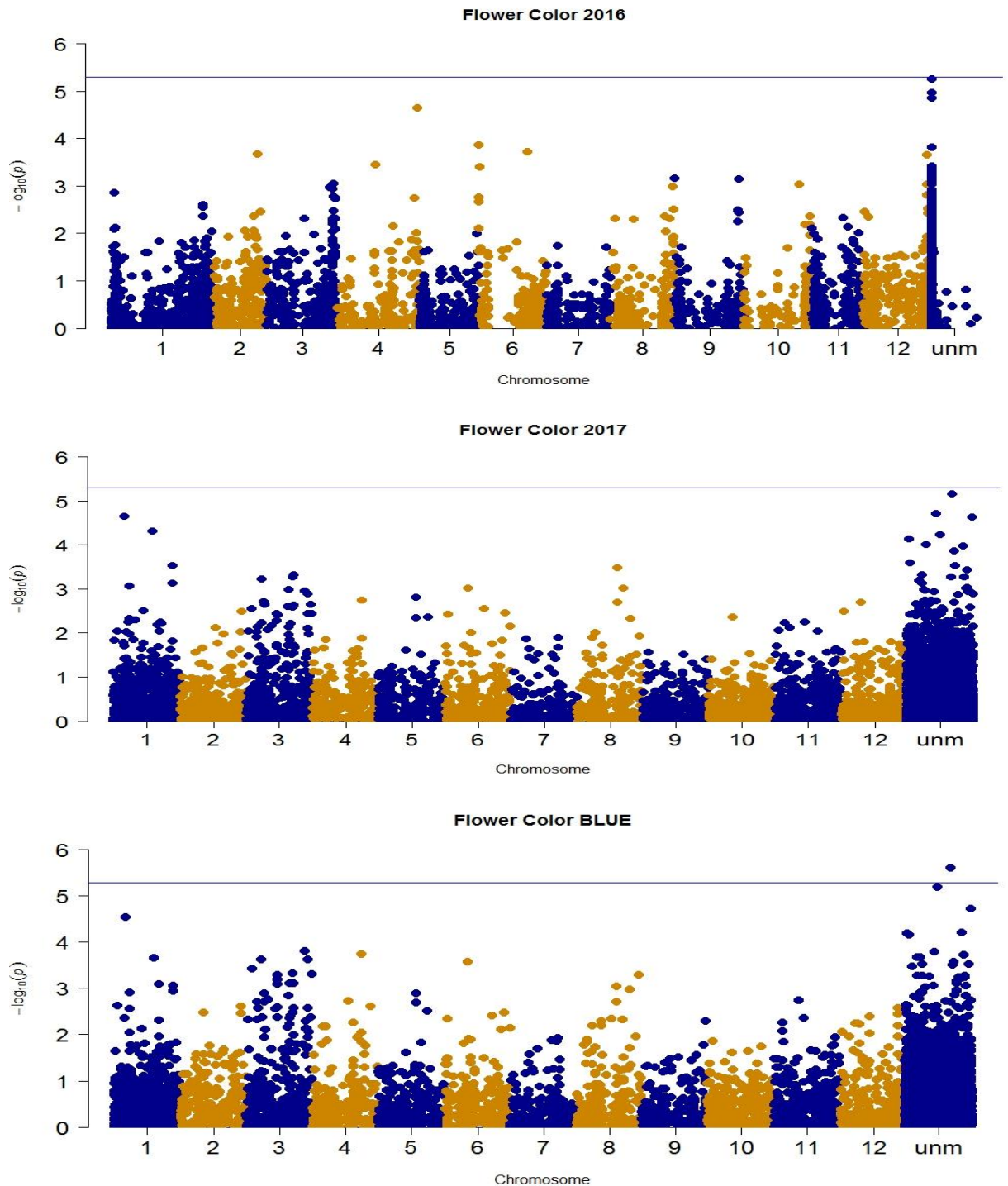


Figure 4.33. Manhattan plots using MLM for Flower color trait in potato for the year 2016, 2017 and BLUE

These results are contradictory to the findings of Berdugo-Cely et al., 2017. They reported that flower color should be on chromosome number 3 with SNP of solcap_snp_c2_36468 but according to our finding it was on chromosome 4 with SNP solcap_snp_c1_13354. In another study conducted by Heckett et al., 2014 reported that the QTL analysis showed the association of flower color at chromosome number 11. We can consider the only marker that we got in our studies for further studies on flower color in potato.

4.5.2.2 Stamen length (SL)

Stamen length of the flowers was measured manually with the help digital compass and was analyzed and found with normal distribution curve over the range of readings that were taken while phenotyping. For validation, either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale or following results were obtained. Q-Q plots showed that the observed p values are the same as the expected p values up to 3 but after that there was some deviations that were observed in each data set. For example, in the data set of 2016, we found that after 3n the observed values are getting fairly below the expected line and the same thing was found for the data set of BLUE. For the data set of year 2017, every observed value was following the expected line except the one point at the end that fell below the expected line from the model that we applied.

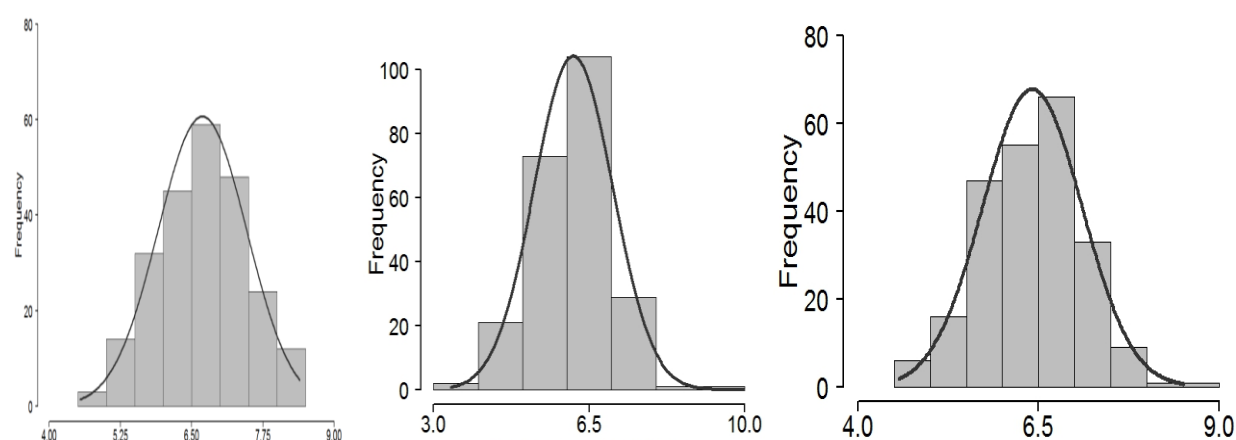


Figure 4.34. Bar plots for the year 2016, 2017 and BLUE for the trait Stamen length

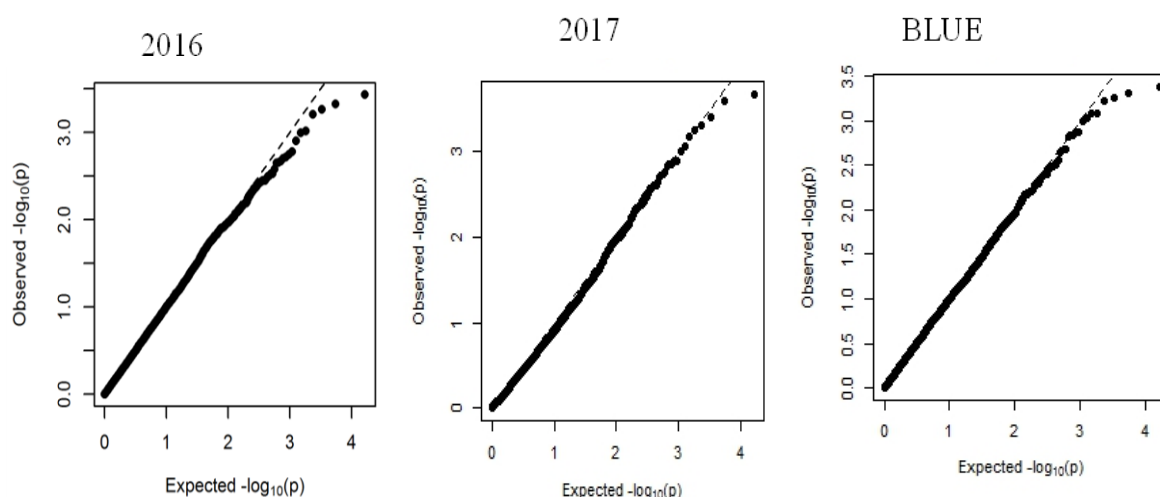


Figure 4.35. Q-Q plots for the trait stamen length in potato plants

After this analysis Manhattan plots were drawn using the p values with the help of statistical software R, and we found that in the data of year 2016, there were some associated markers that were associated with Stamen length of the potato plants (Table 4.15).

Table 4.15. SNP found in 2016 data set from GLM for stamen length

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_25692	3	Pentatricopeptide repeat-containing protein At3g49240	2612	3.66E-07
solcap_snp_c2_25723	3	Metal-nicotianamine transporter YSL3	2614	8.24E-07
solcap_snp_c1_14701	5	rRNA methyltransferase 1	5358	2.70E-06
solcap_snp_c2_9189	6	RPM1-interacting protein 4	4588	2.74E-06
solcap_snp_c2_42765	7	C2 domain-containing protein At1g53590	3493	2.84E-06
solcap_snp_c2_15660	5	Uncharacterized	2142	4.70E-06

Contrastingly, in the data of year 2017, there was no SNP marker that was found to be associated with Stamen length of flower, not even unmapped markers. In the Manhattan plots of BLUEs data, we found a couple of SNPs associated with Stamen length but

those were among the unmapped markers. On chromosome 1 and 3 there were some markers that were very near to the threshold line but not associated with the traits.

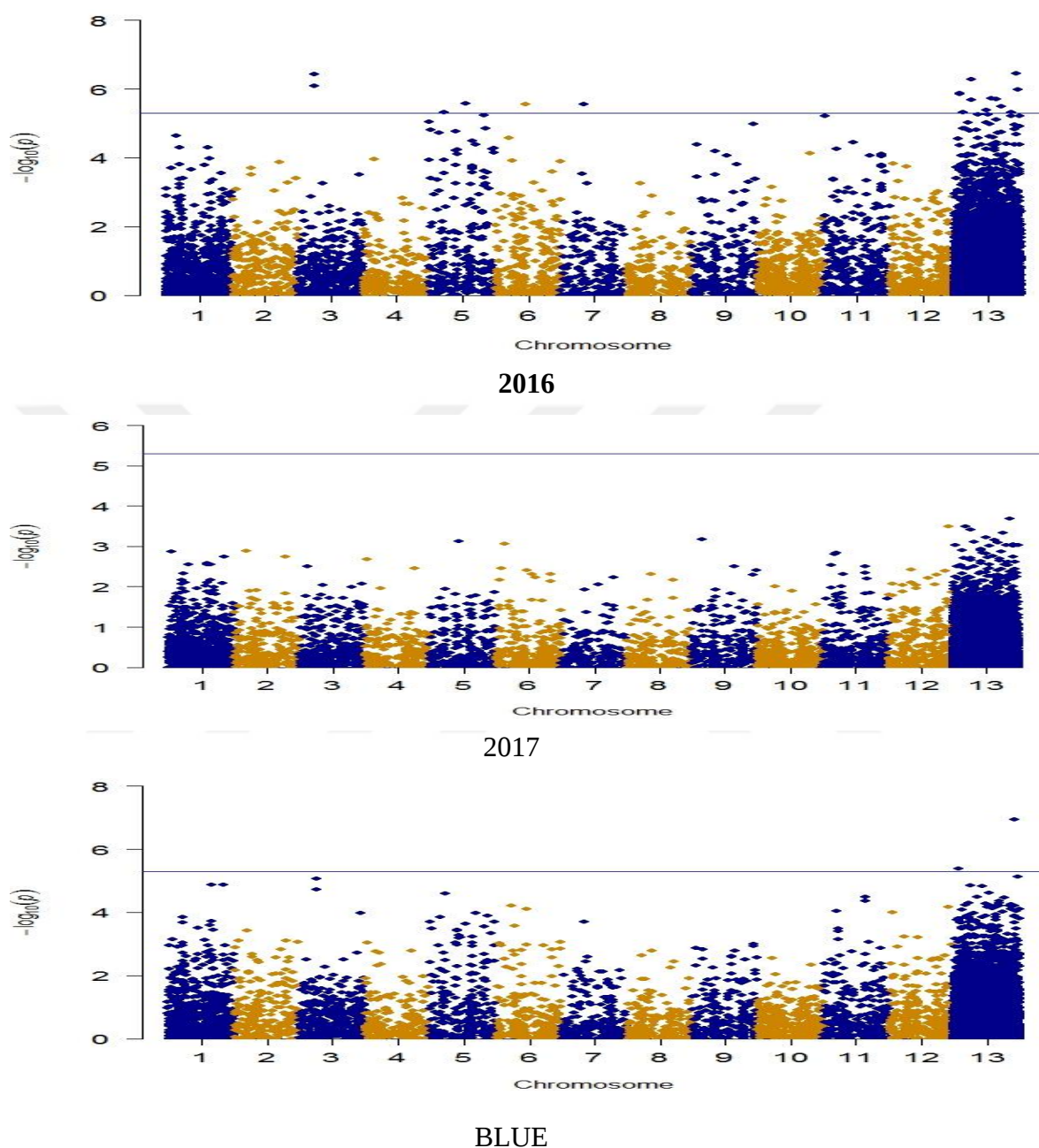


Figure 4.36. Manhattan plots using Generalized Linear Model for Stamen length trait in potato

MLM was applied to all data sets and following results were found, 3 SNP markers on chromosome 3 and 6 were found to be associated with Stamen length when MLM was applied to the data set of year 2016 data. However, there was no significantly associated marker in other data sets that are 2017 and BLUEs.

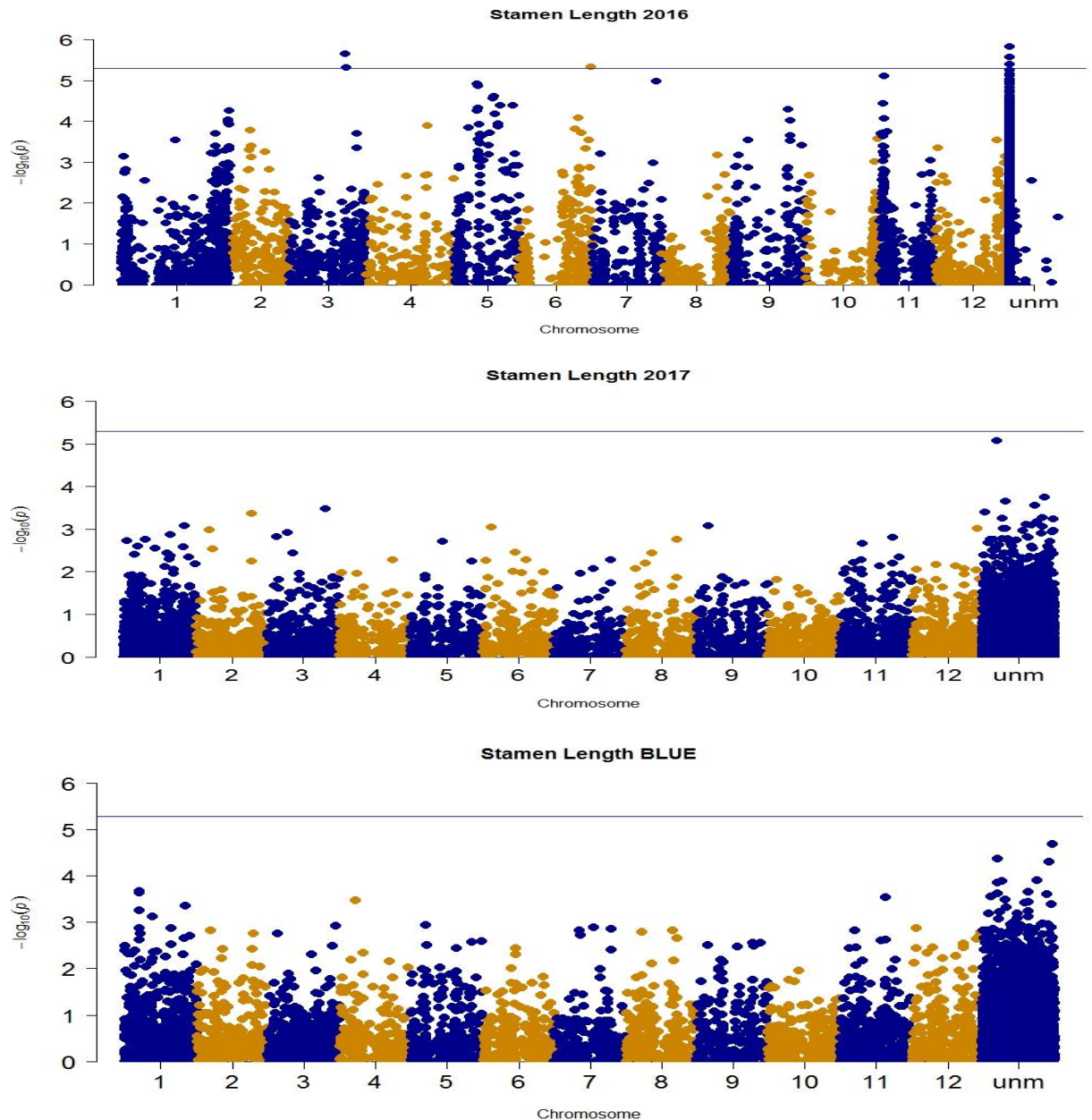


Figure 4.37. Manhattan plots using MLM for Stamen length trait in potato

Li et al., 2001 while studying QTL analysis of Stamen length and ratio of stigma exertion, two key traits of classification for cultivated rice (*Oryza sativa* L.) and common wild rice (*O. rufipogon* Griff.) described that QTLs controlling Stamen length located at the region of C424-G39 of Chromosome 2, and C2807-C1263 of Chromosome 9 respectively. QTLs controlling Stamen length located at the region of C424-G39 of Chromosome 2, and C2807-C1263 of Chromosome 9 respectively. We found some significant associations on chromosome number 3, 5, 6 and 7. We can consider the markers on chromosome 3 (solcap_snp_c2_25692 and

solcap_snp_c2_25723) and 6 (solcap_snp_c2_9189) for future studies of stamen length in potato as these were found common in both the models.

4.5.2.3 Pistil length (PL)

Pistil length of the flowers was measured manually with the help digital compass and was analyzed and found with normal distribution curve over the range of readings that were taken while phenotyping. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot representing expected versus observed P values at $-\log_{10}$ scale and following results were obtained.

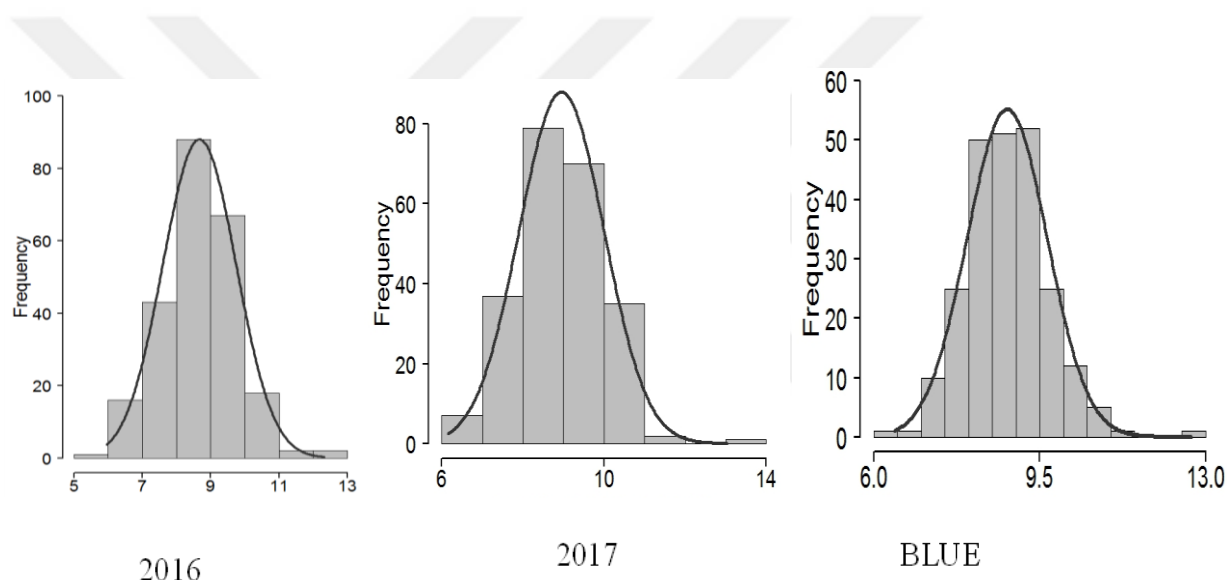


Figure 4.38. Bar plots for the trait Pistil length for the growing season of 2016, 2017 and BLUE

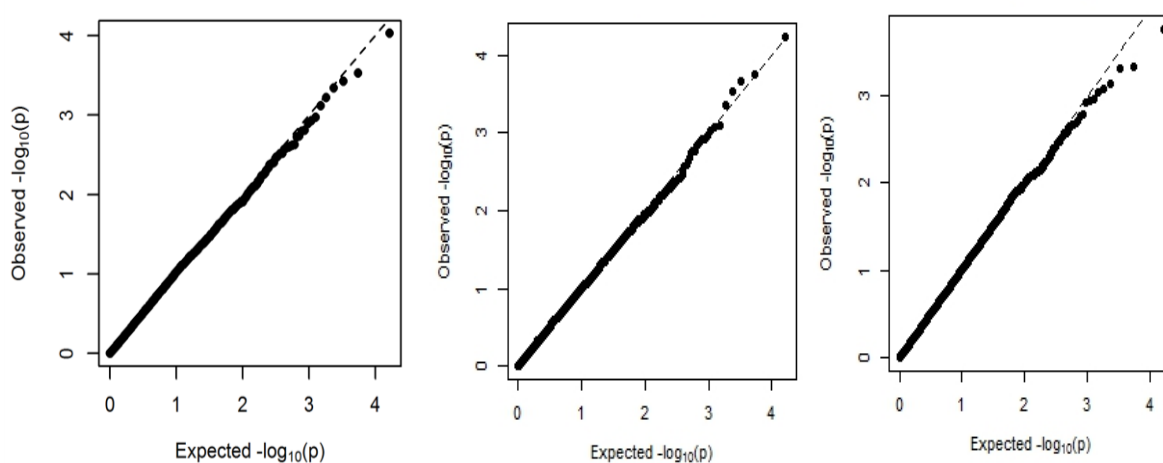


Figure 4.39. Q-Q plots for the trait Pistil length for 2016, 2017 and BLUE

Obtained Q-Q plots showed fairly the similar results following the expected values for Pistil length that our model gave. Graphs for each data set was found normal as per expected value but for the data set of BLUE, we found that at the end around 4 to 5 points were there that deviated below from the expected line.

After Q-Q plots, Manhattan plots were drawn to see either there is some associations among the trait and the SNPs or not. Manhattan analysis of Pistil length showed the results like,

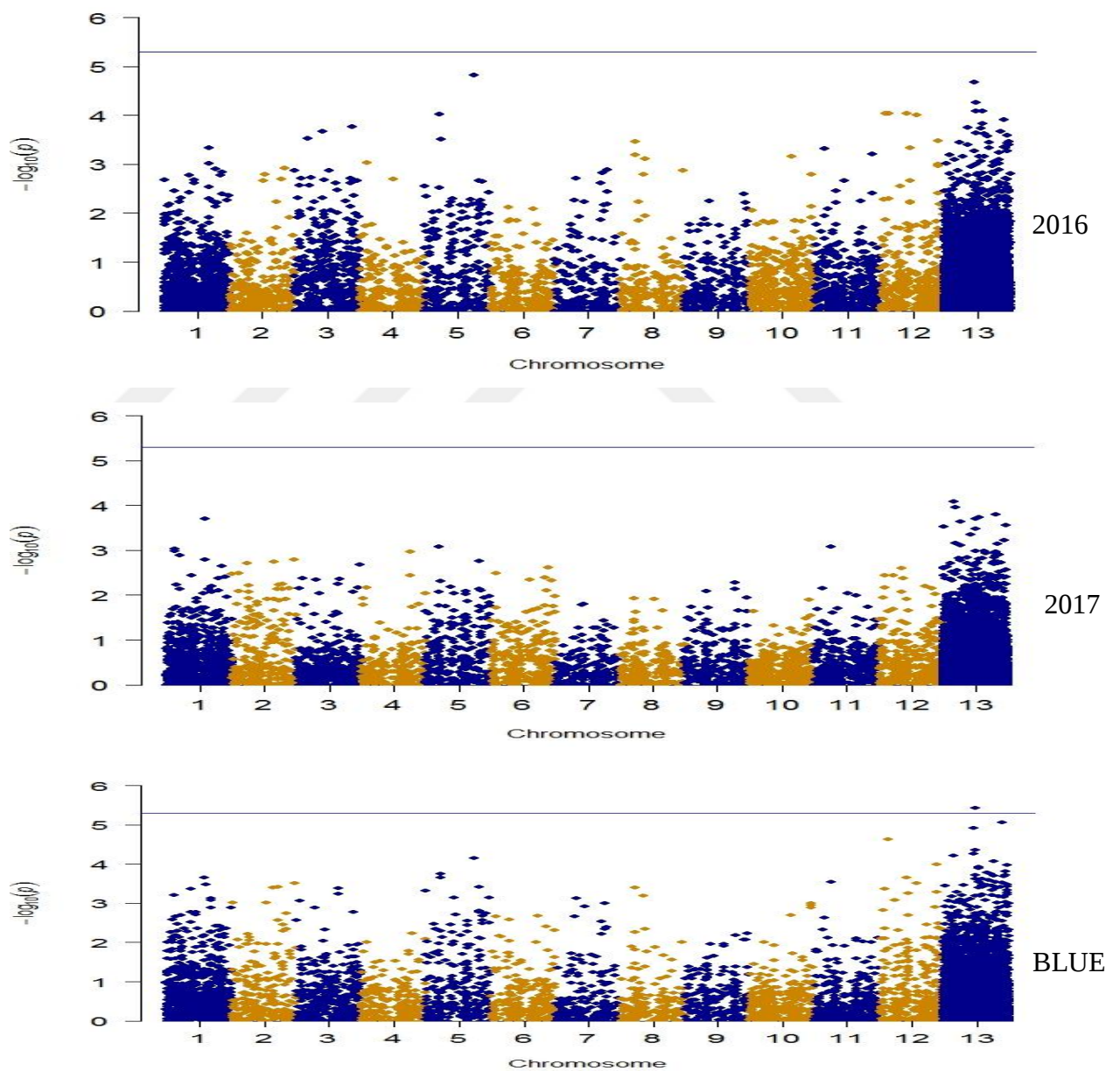


Figure 4.40. Manhattan plots using Generalized Linear Model for Pistil length trait in potato

After getting Manhattan plots we found that there was no SNP marker associated with Pistil length of potato flower. Only in BLUE, one marker was associated with this trait but that was among the unmapped markers. After GLM analysis, we performed MLM analysis for our data sets and not even a single SNP marker was found to be associated with length of stigma.

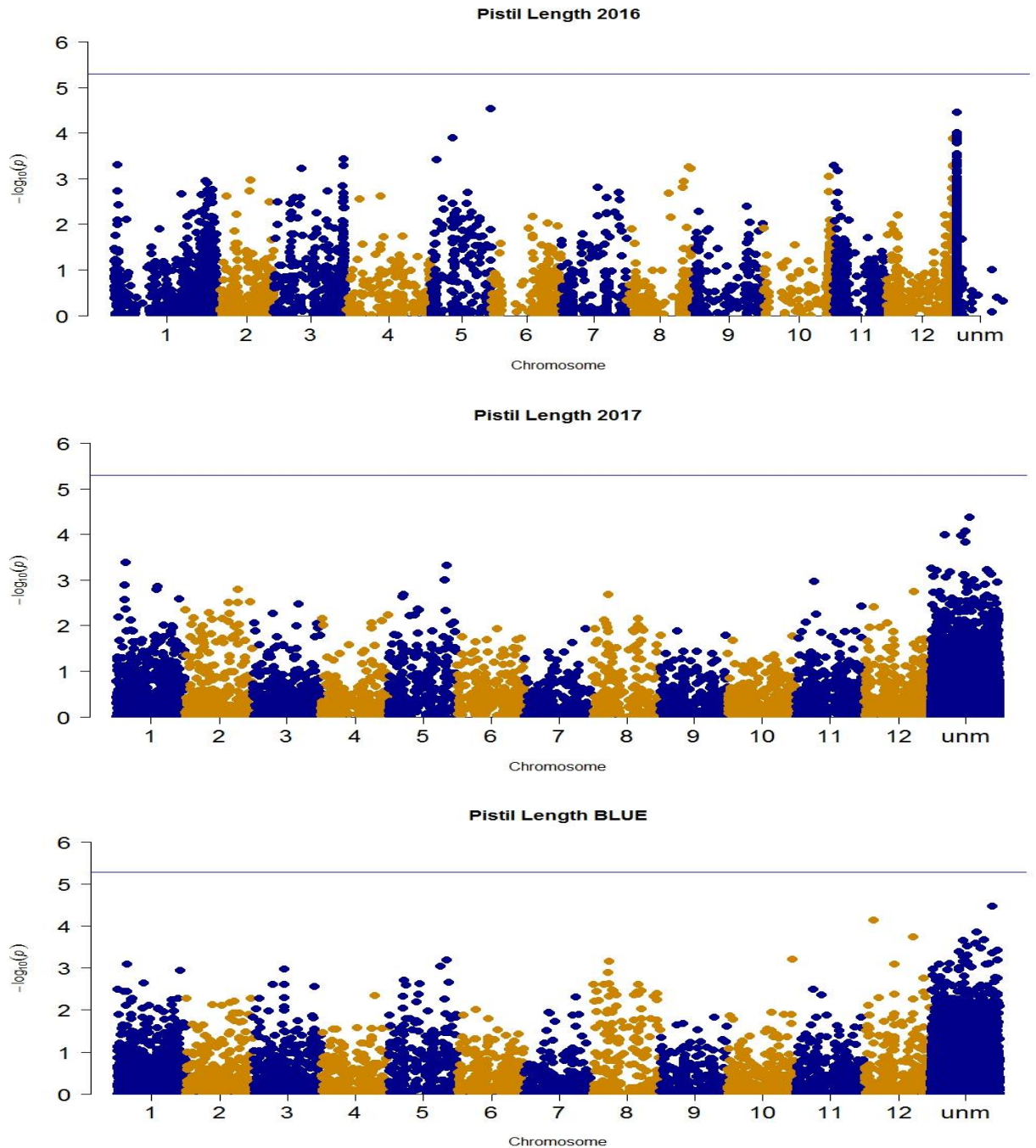


Figure 4.41. Manhattan plots using Mixed Linear Model for Pistil length trait in potato

Liu et al., 2015, while studying Pistil length in rice described that Pistil length is linked to the short arm on chromosome number 3 of rice. While for our studies we were unable to find any significant association on any chromosome specifically for the trait of Pistil length.

4.5.2.4 Pistil length Above Stamen (PLAS)

Pistil length above stamen of the flowers was measured manually with the help digital compass and was analyzed and found with normal distribution curve over the range of readings that were taken while phenotyping. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot representing expected versus observed P values at $-\log_{10}$ scale and following results were obtained. Q-Q plots showed that the observed p values are the same as the expected p values but for the data set of year 2016 there were two points that were found above of the expected line and for the data set of year 2017 and BLUEs, there was one point each that was found below the expect p value line for this specific trait.

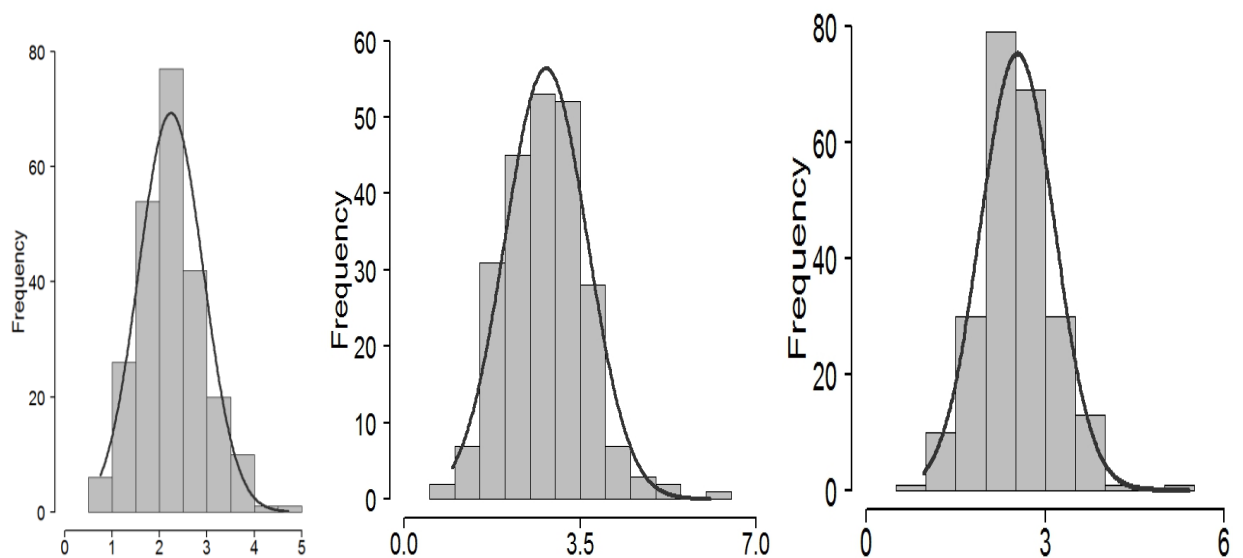


Figure 4.42. Bar plots for the trait Pistil length above stamen for potato plants

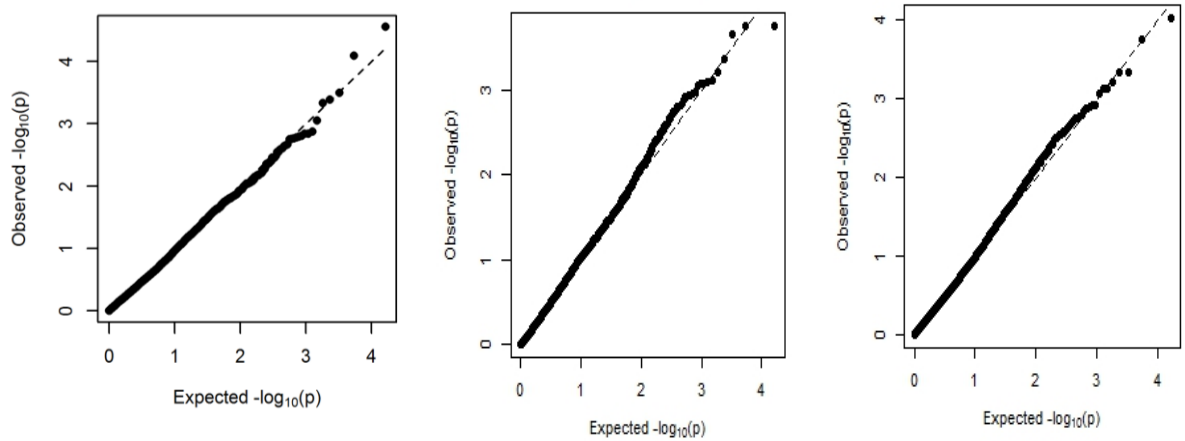


Figure 4.43. Q-Q plots for the trait Pistil length above stamen for potato plants

Manhattan plots were drawn against the p values and got the following results. In all the data sets there was no marker that was associated with the specific trait of the plant. However, there were some markers that were associated but those were among unmapped markers. In 2016 data on chromosome 4 there was a SNP marker that was very near to the threshold level but was not above the line to be associated with the Pistil length above anthers.

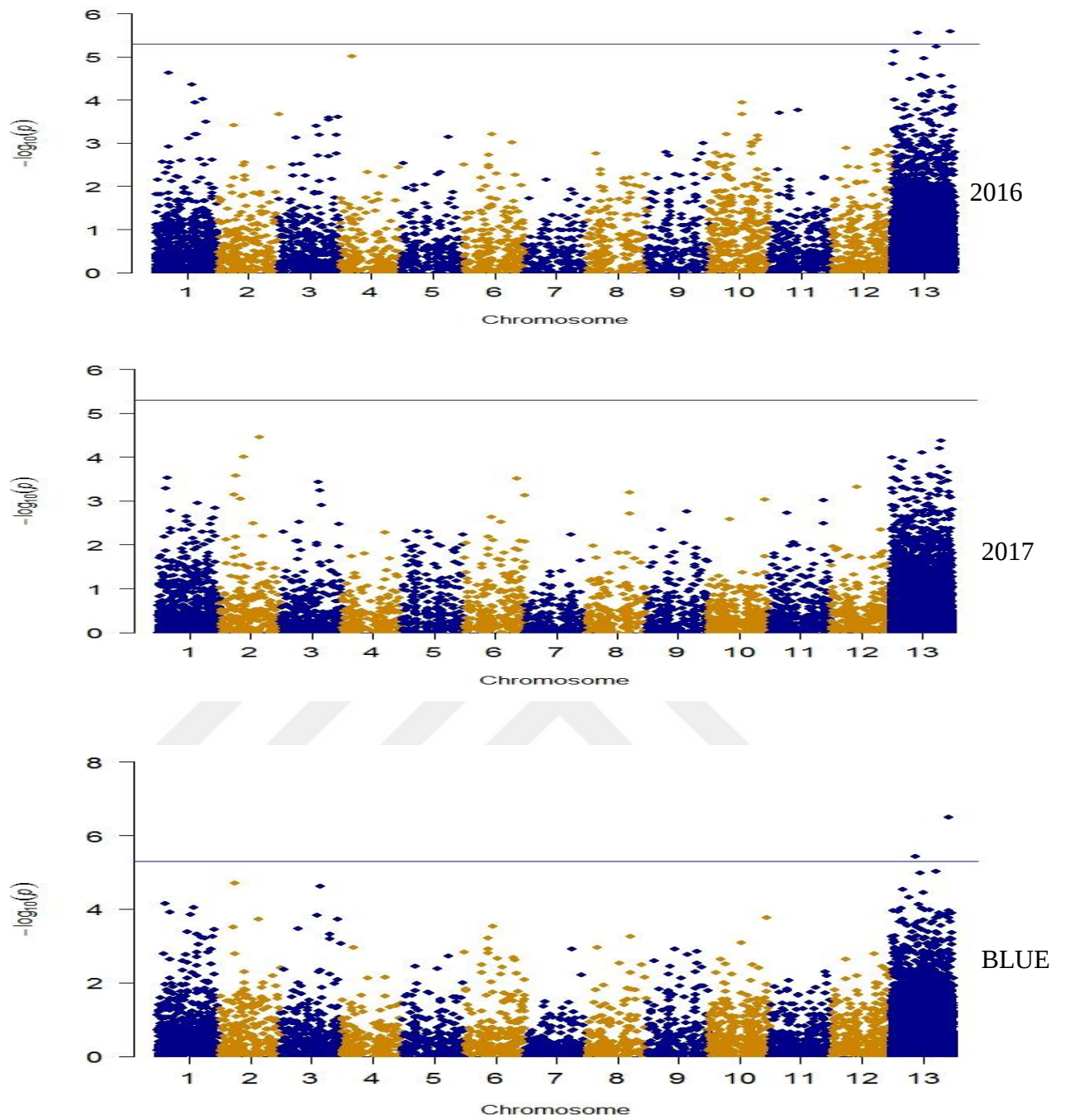


Figure 4.44. Manhattan plots using Generalized Linear Model for Pistil length above stamen trait in potato

After GLM analysis, we performed MLM analysis for our data sets and not even a single SNP marker was found to be associated with length of stigma.

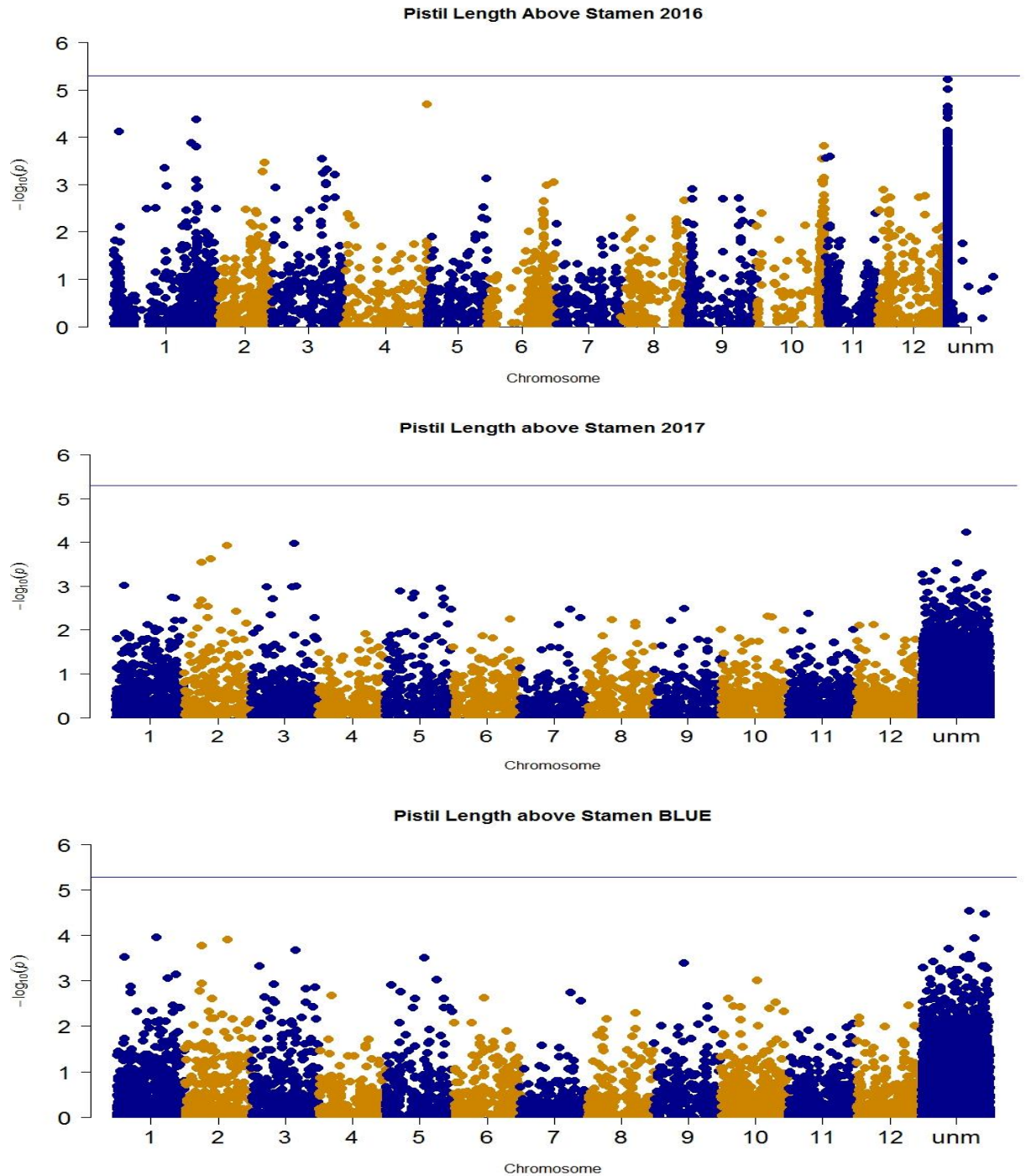


Figure 4.45. Manhattan plots using Mixed Linear Model for Pistil length above stamen trait in potato

Li et al., 2001 while studying QTL analysis of Stamen length and ratio of stigma exertion, two key traits of classification for cultivated rice (*Oryza sativa* L.) and common wild rice (*O. rufipogon* Griff.) described that there are two QTLs controlling ratio of stigma exertion and located at the region of C2289-R1553 of Chromosome 5 and G1149-R1963 of Chromosome 8 respectively. But unfortunately, we were unable to find any significant associated marker at any chromosome in our parameters.

4.5.3 Tuber Traits

4.5.3.1 Tuber Skin Color (TSC)

Tuber Skin Color was observed and was then analyzed and found with normal distribution curve over the range of readings that were taken while phenotyping. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot representing expected versus observed P values at $-\log_{10}$ scale and following results were obtained. Q-Q plots showed that the observed p values are the same as the expected p values but at the end of data set of 2016 and 2017 there was a point in both graphs that went a bit below than the models' expected p value line. But for the data set of BLUE, we found that there was one point that whose value was going above of the expected p value line.

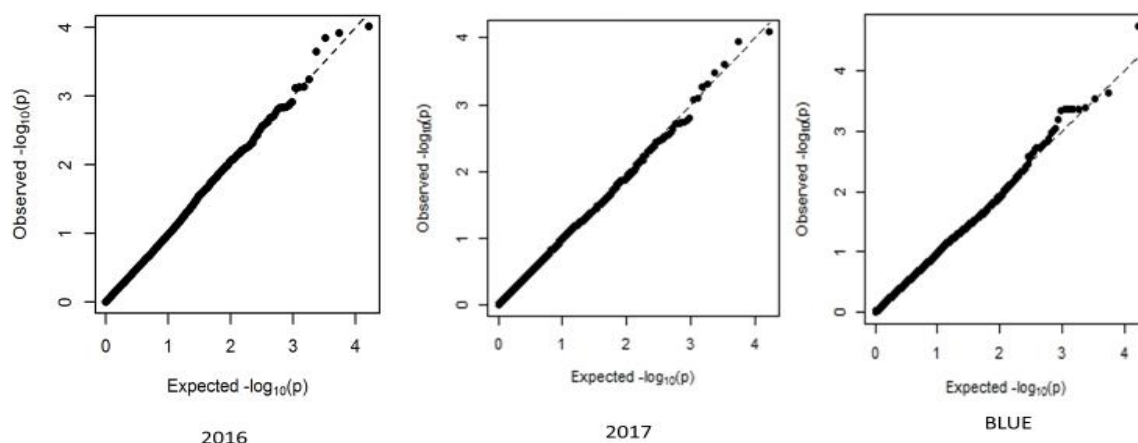


Figure 4.46. Q-Q plots for 2016, 2017 and BLUE for tuber skin color in potato

Manhattan plots for tuber skin color were drawn using the p values that we got from TASSEL and got the following results. In the data of year 2016, we found that 06 SNP markers were significantly associated with tuber skin color. From that two of them were among unmapped markers and others were located on chromosome 3 and 4 (Table 4.16).

Table 4.16. SNP found in 2016 data set from GLM for tuber skin color

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_247	3	Protein BONZAI 1, Transcript Variant	6819	1.87E-06
solcap_snp_c2_38730	4	sodium/hydrogen exchanger	3304	3.65E-06
solcap_snp_c2_244	3	Protein BONZAI 1, Transcript Variant	2560	3.82E-06
solcap_snp_c2_295	3	Poly [ADP-ribose] polymerase 1	7045	4.55E-06

For the data of year 2017 only one SNP marker was significantly associated with tuber skin color of potato crop (Table 4.17).

Table 4.17. SNP found in 2017 data set from GLM for tuber skin color

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_19252	1	Thioredoxin X	2319	4.77E-06

For the data of BLUEs, there were two markers that were associated with tuber skin color. One of them was among unmapped markers and the other was on chromosome 1 (Table 4.18).

Table 4.18. SNP found in BLUE data set from GLM for tuber skin color

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_19252	1	Thioredoxin X	2319	3.43E-06

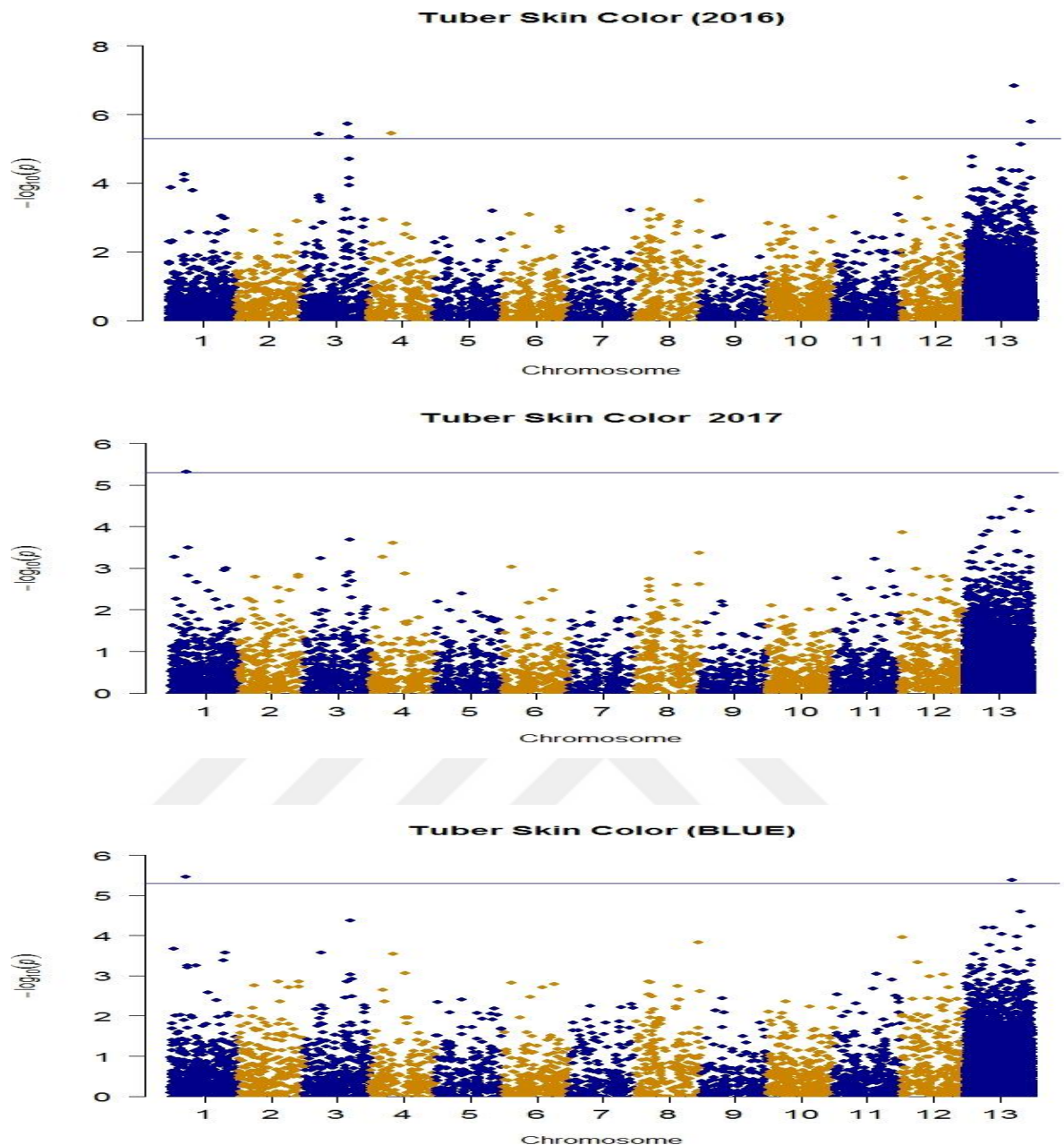


Figure 4.47. Manhattan plots using Generalized Linear Model for tuber skin color trait in potato

It was noted that in the Manhattan of years 2017 and BLUEs on chromosome 1 same SNP marker was significantly associated with tuber skin color of potato crop that was solcap_snp_c2_19252.

After analyzing data from GLM, we also performed mixed linear model and found following results, For the case of tuber skin color there was some significantly associated markers on chromosome 3 except unmapped for the data set of 2016. While

for the data sets of 2017 and BLUEs there was no SNP marker that was associated to the trait (Table 4.19).

Table 4.19. SNP found in 2016 data set from MLM for tuber skin color

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_247	3	Protein BONZAI 1, Transcript Variant	57872050	2.15E-06
solcap_snp_c2_295	3	Poly [ADP-ribose] polymerase 1	57635783	3.81E-06

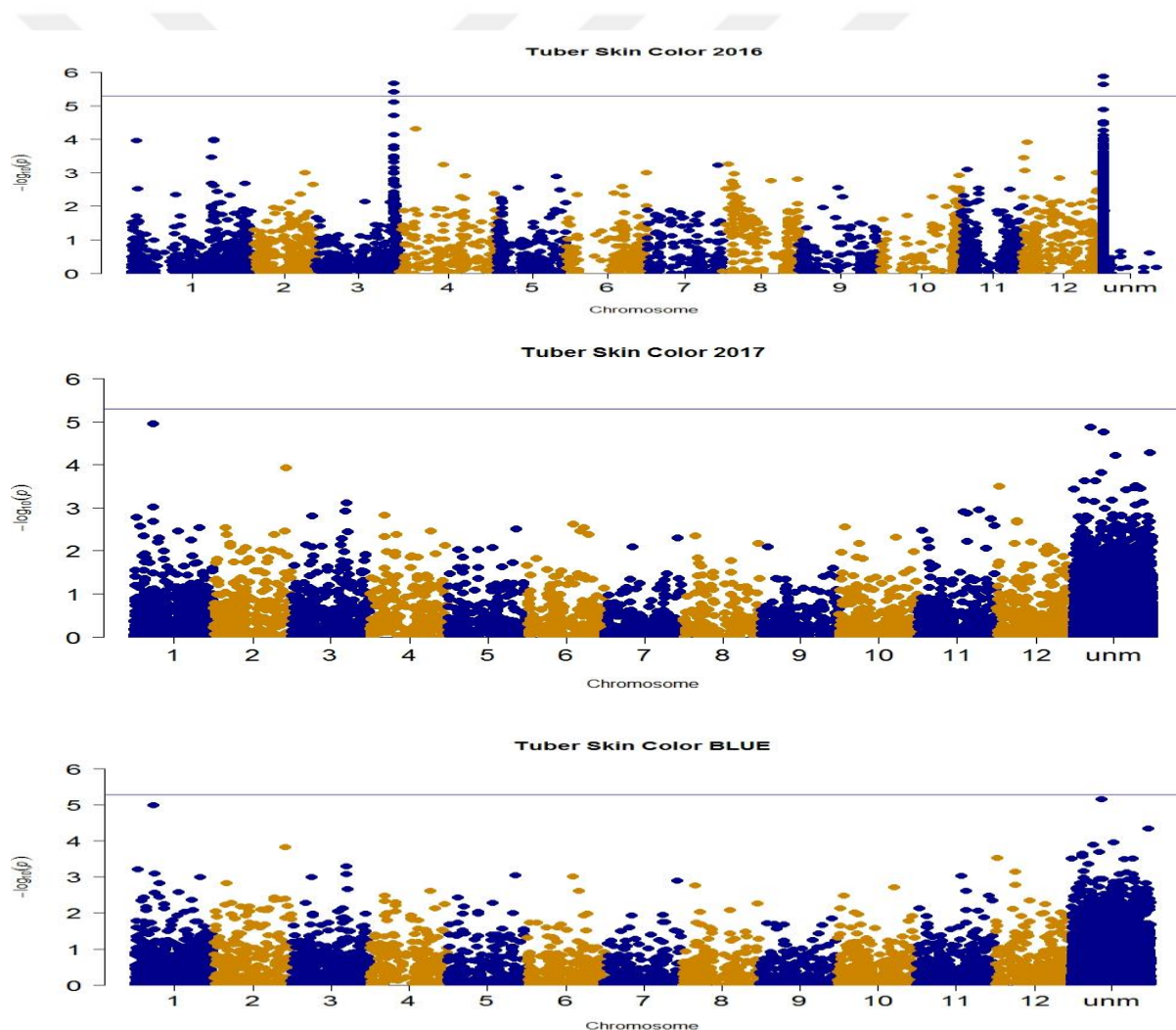


Figure 4.48. Manhattan plots using Mixed Linear Model for tuber skin color trait in potato

The results of our study were in contrast with the findings of Berdugo-Cely et al., 2017 who reported that it should be linked on chromosome number 10 with the SNP of solcap_snp_c2_45235, but in our studies it was found on different chromosome number majorly on chromosome 1 and 3 and also in one case it was found to be on chromosome 4. In tuber skin color of potato the markers that can be considered for future studies in potato are on chromosome 3 (solcap_snp_c2_247, solcap_snp-c2_295) as these were found common in both the models.

4.5.3.2 Number of Tubers per Plant (NTP)

Number of tuber per plant was counted and then analyzed for the association mapping studies. In bar graphs we observed some abnormal distribution over the years for example in data set of year 2016 the curve was starting from the middle while for the year 2017 the curve was even more higher than 2016 and was starting near around frequency level of 20. For validation either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot representing expected versus observed P values at $-\log_{10}$ scale and following results were obtained. Q-Q plots showed that the observed p values are the same as the expected p values. Due to abnormalities found in bar graphs resulting qq plots obtained were also not ideal and showed some deviation. In the data set of year 2016 it was forming a curve from the middle of the graph. While in the case of year 2017 data set the graph was not obtained smooth it was not smooth from middle and same is the case was found in BLUE that showed some abnormalities compared to the ideal curve. This could be due to the reason of environment and phenotype interactions that affected formation of number of stems that

ultimately caused the number of tubers per plant.

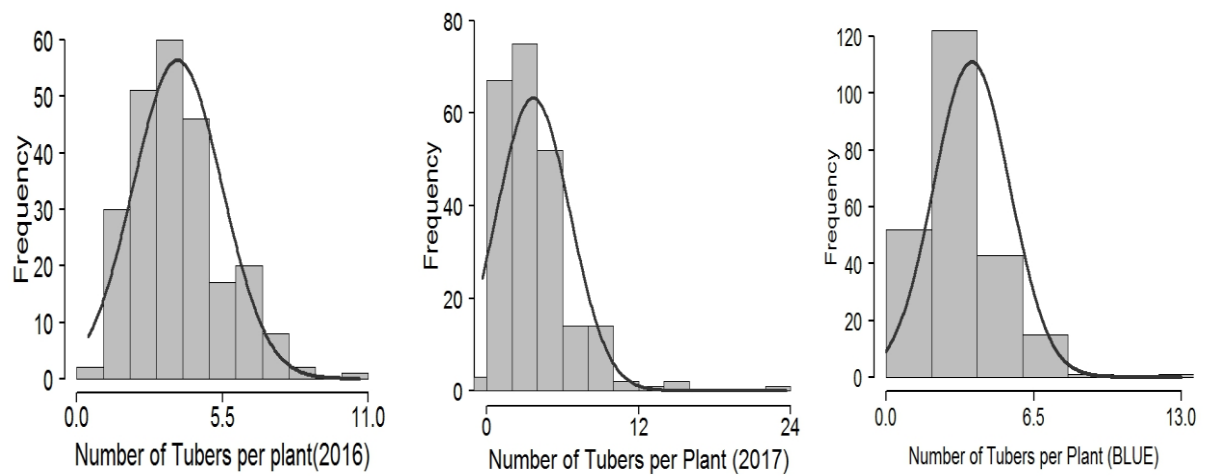


Figure 4.49. Bar plots for number of tubers per plant for the year 2016, 2017 and BLUE

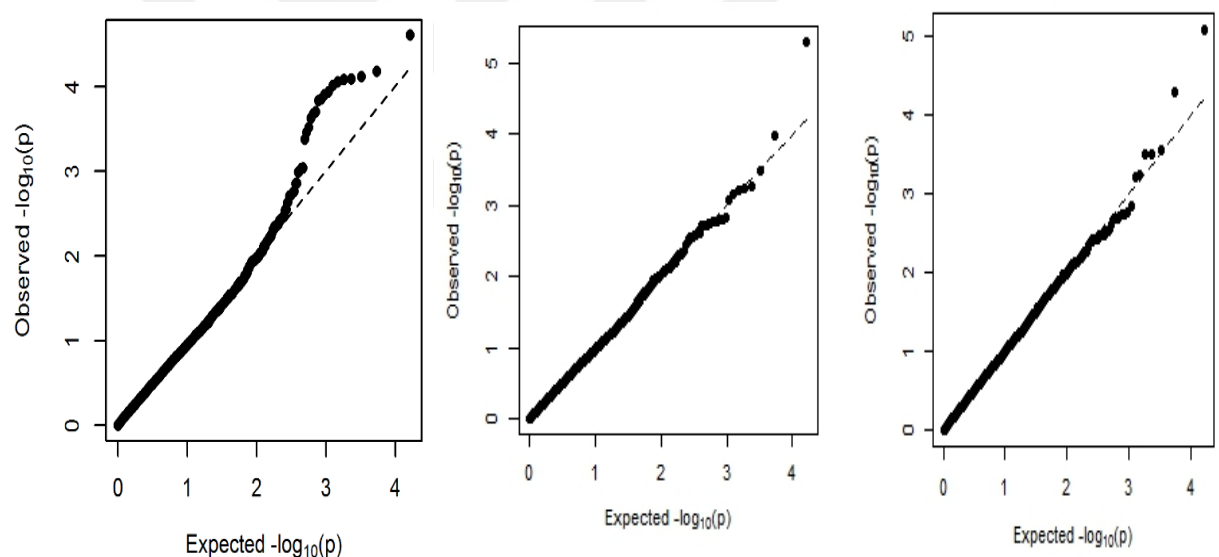


Figure 4.50. Q-Q plots for number of tuber per plant traits in potato for the year 2016, 2017 and BLUE

Manhattan plots were drawn after these analyses and found the results like, For Manhattan plot of 2016 year data, there were some SNPs that were significantly associated with number of tubers per plant (Table 4.20).

Table 4.20. SNP found in 2016 data set from GLM for number of tubers per plant

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_29272	7	Protein WEAK CHLOROPLAST MOVEMENT UNDER BLUE LIGHT	7022	2.92E- 06

All the associated SNPs were among the unmapped markers except one that was associated on chromosome number 7. On chromosome 1, there was a marker that was found to be very close to the threshold line. For the Manhattan that we got for the year 2017 data, only one SNP was associated with the trait that was present on chromosome 1 (Table 4.21).

Table 4.21. SNP found in 2017 data set from GLM for number of tubers per plant

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_6965	1	lignin-forming anionic peroxidase	9767	1.91E-06

There were two SNP markers that were associated with number of tuber per plant on chromosome 1 (Table 4.22).

Table 4.22. SNP found in BLUE data set from GLM for number of tubers per plant

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_36675	1	Ankyrin repeat-containing protein At2g01680	3197	1.43E-06
solcap_snp_c2_6965	1	lignin-forming anionic peroxidase	9767	1.80E-06

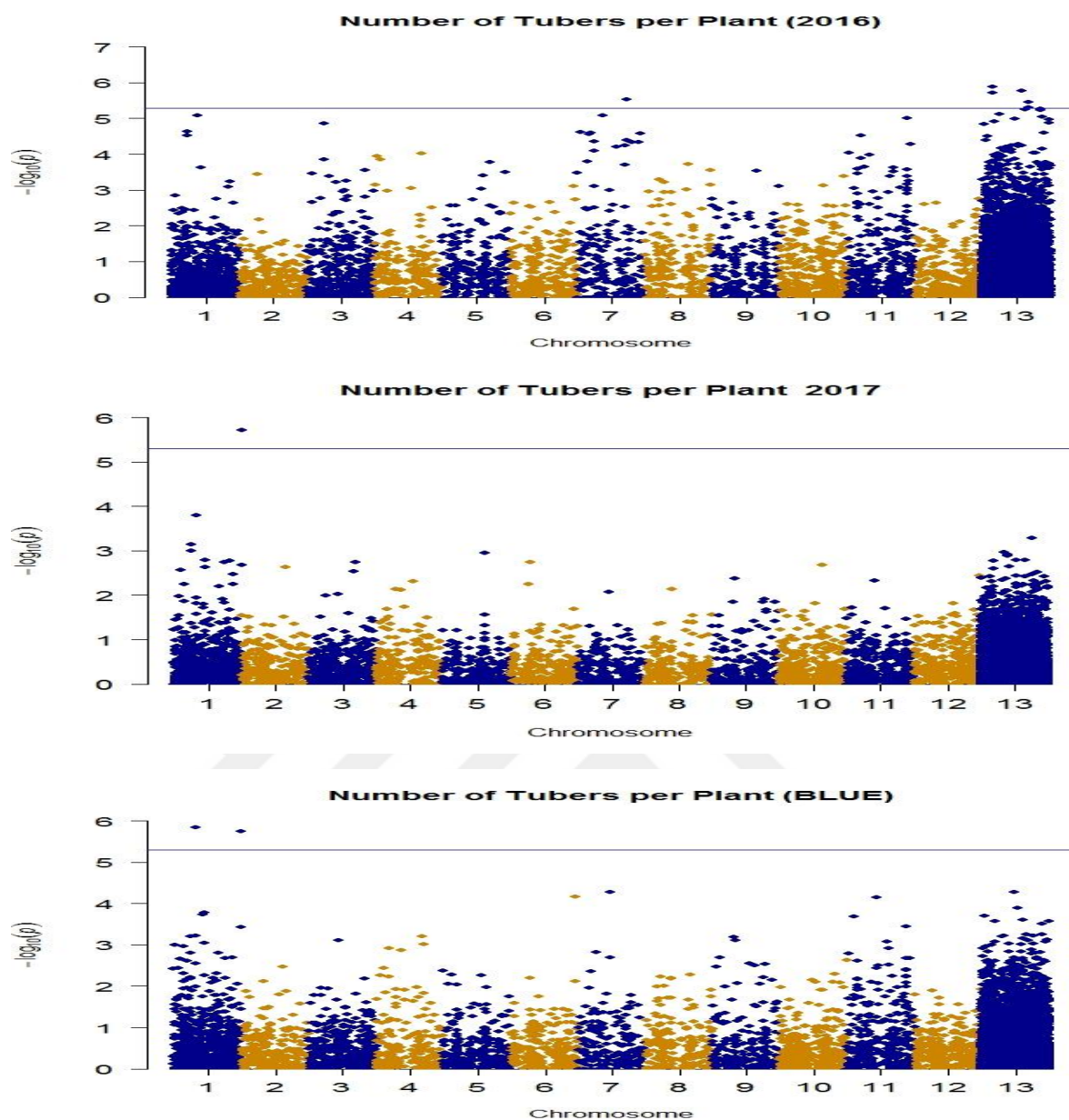


Figure 4.51. Manhattan plots using Generalized Linear Model for number of tuber per plant trait in potato for the year 2016, 2017 and BLUE

Here in number of tuber per plant for 2017 and BLUEs, solcap_snp_c2_6965 was a common SNP that was associated specifically with this trait. After GLM, we performed MLM to our data and found the following results, markers were found to be associated with number of tubers per plant for the data set of 2016 (Table 4.23).

Table 4.23. SNP found in 2016 data set from MLM for number of tubers per plant

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_46573	7	Uncharacterized	24186425	1.46E-06
solcap_snp_c2_49473	1	Probable purine permease 11	68400084	3.39E-06
solcap_snp_c2_24373	5	Two-component response regulator ARR11	11544170	3.99E-06
solcap_snp_c2_29254	7	Chlorophyll a-b binding protein P4	31605268	4.17E-06
solcap_snp_c2_47917	8	Beta-galactosidase	7860923	4.23E-06

No SNP marker was found to be associated in 2017 and BLUEs data set.

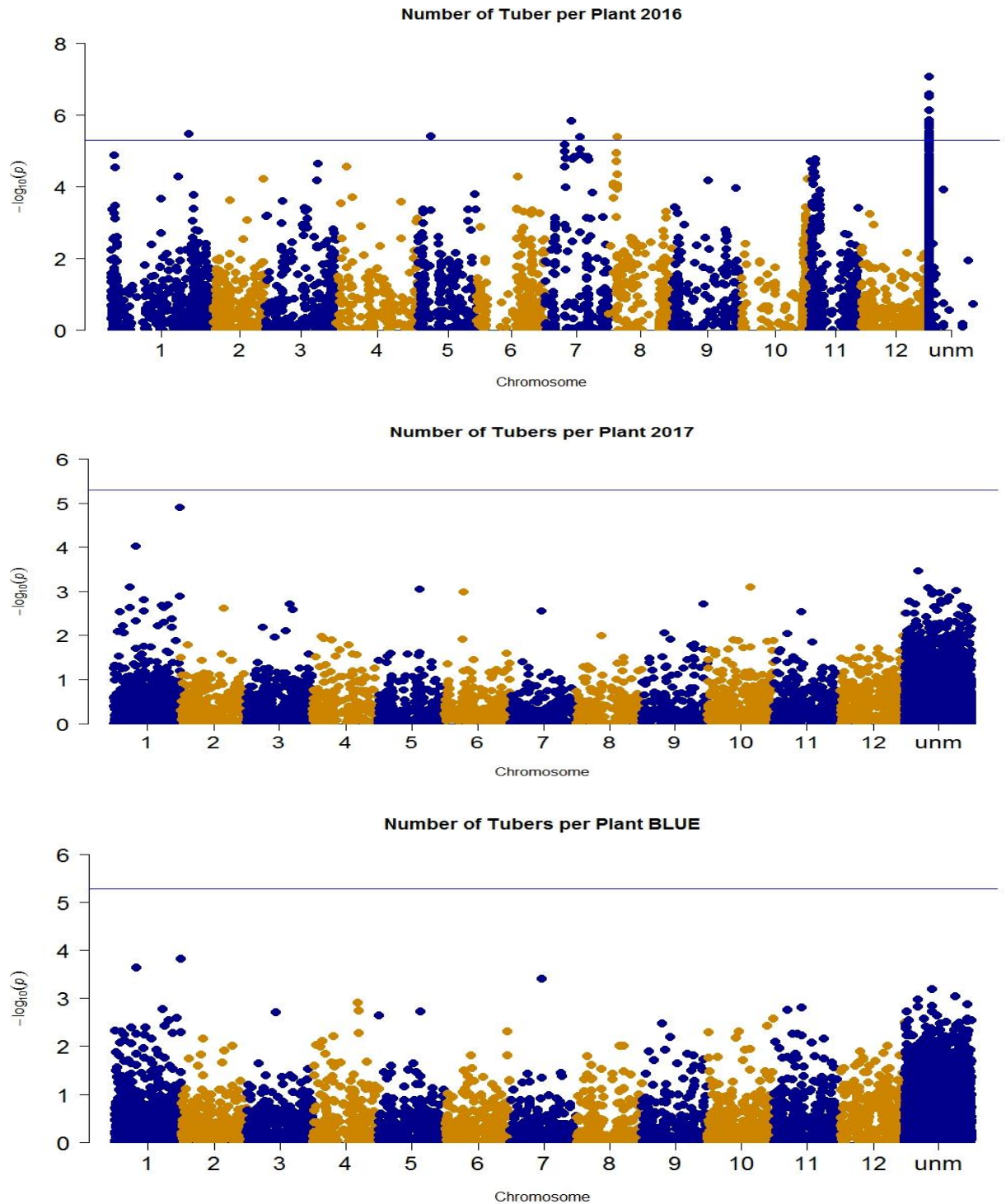


Figure 4.52. Manhattan plots using MLM for number of tuber per plant trait in potato

These results are in accordance with the findings of Manrique-Carpintero et al., 2015, who reported by utilizing QTL studies on diploid potato species that number of tubers per plant/ tuber set is associated on chromosome number 5. Our findings were also in accordance with the findings of Manrique-Carpintero et al., 2005, we also found that one of the SNP is linked to chromosome number 5 and but also chromosome 1, 7 and 8

were found to be associated with number of tubers per plant. We can consider the marker on chromosome 1 (solcap_snp_c2_6965) for further studies as it was found common in 2017 and for BLUE in generalized linear model.

4.5.3.3 Tuber Shape (TS)

Tuber Shape was observed by their physical appearance and the readings were recorded. Bar graph analysis showed normal distribution curve over the range of readings that were taken while phenotyping. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot representing expected versus observed P values at $-\log_{10}$ scale and following results were obtained. Q-Q plots showed that the observed p values are the same as the expected p values.

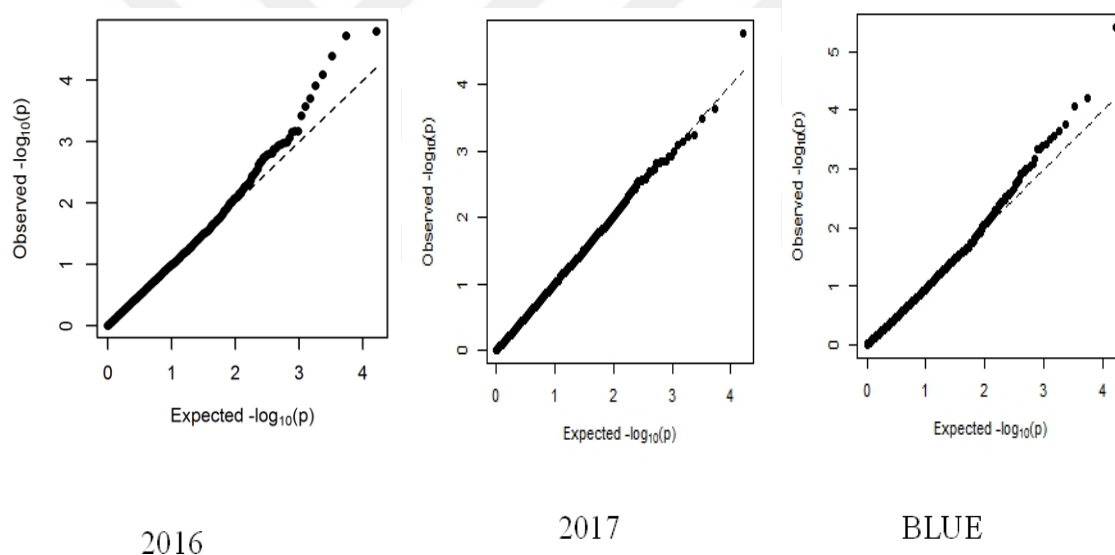


Figure 4.53. Q-Q plots for tuber shape in potato for the year 2016, 2017 and BLUE

Q-Q plot for the year 2016 showed a bit increase at the end of the graph while for 2017 it was same as the expected values for tuber shape. In case of BLUEs, the observed values were a bit at higher node as per expected ones.

Manhattan plots were drawn for the p values of tuber shape and observed that except unmapped marker no SNP was significantly associated for the trait of tuber shape for the data of year 2016.

For the Manhattan plots of 2017 and BLUEs value there was nothing that was associated with tuber traits. Not even any marker from unmapped ones was found to be associated with the trait.

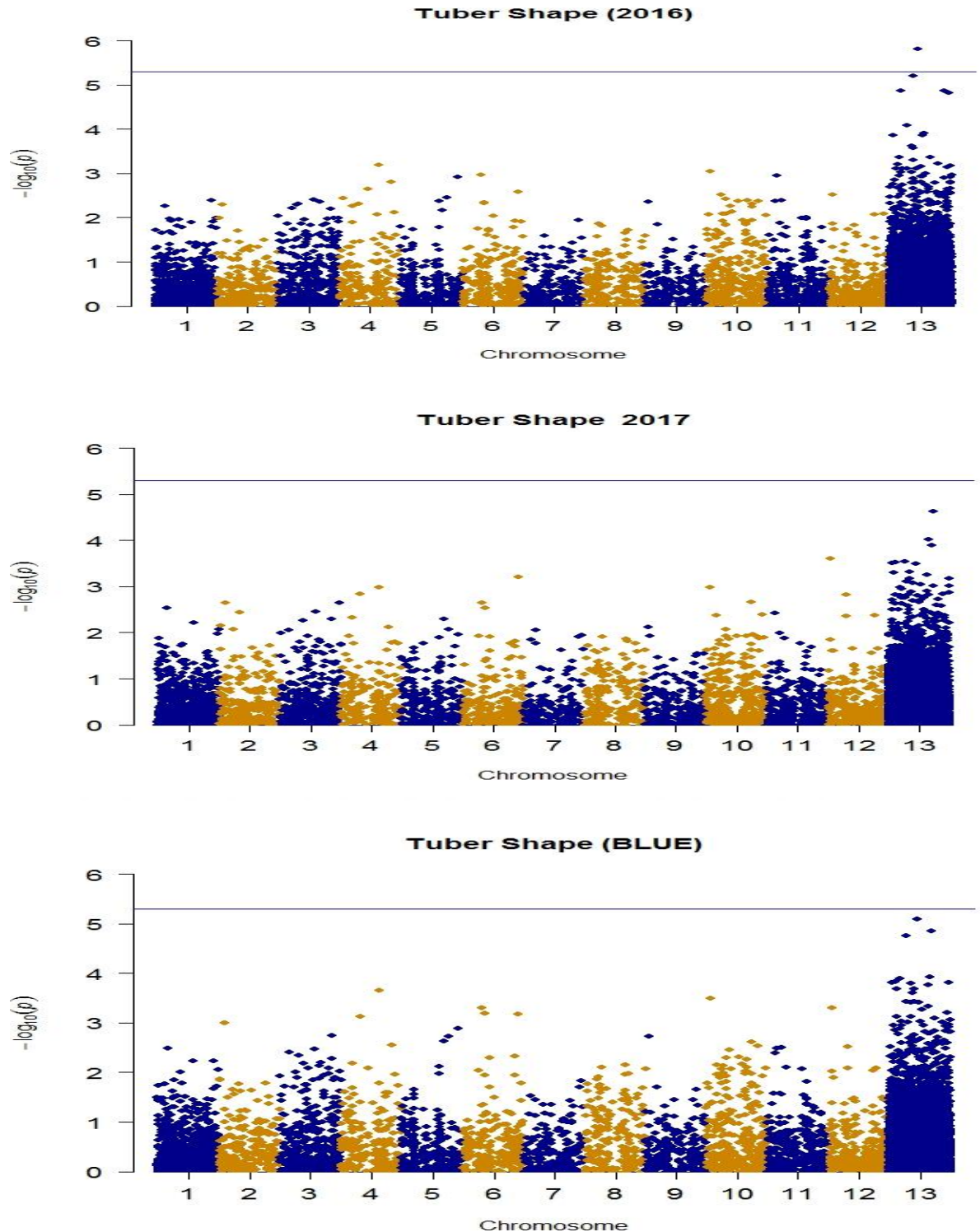


Figure 4.54. Manhattan plots using Generalized Linear Model for tuber shape trait in potato

After analyzing the results with GLM, we also performed Mixed Linear Model (MLM) to our data and surprisingly there was no SNP associated to the tuber shape of the potato crop. All the SNPs were below the threshold level showing no association to the trait.

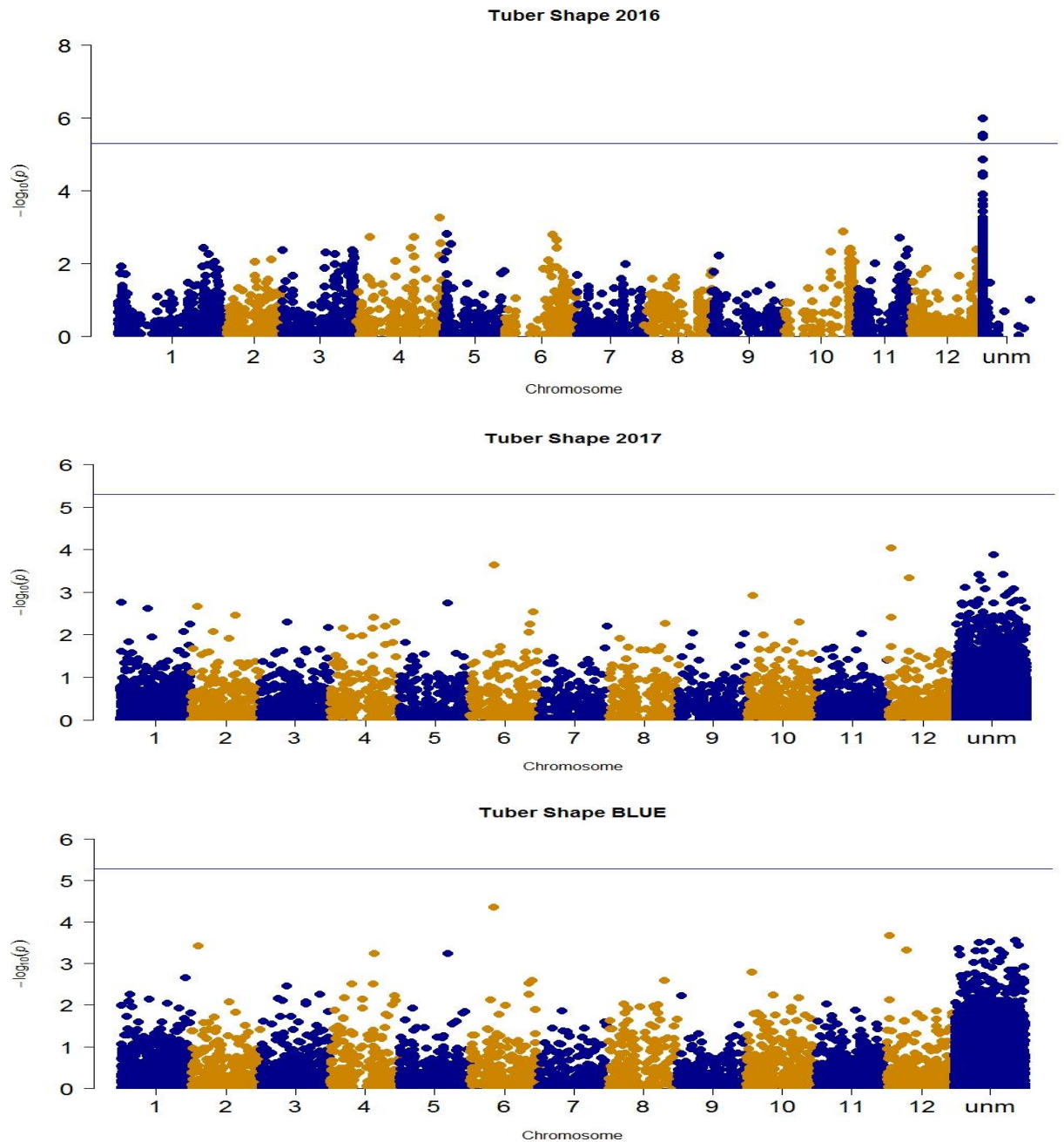


Figure 4.55. Manhattan plots using Mixed Linear Model for tuber shape trait in potato

These findings were not according to the findings of Berdugo-Cely et al., 2017 and Van Eck et al., 1994 who reported that the trait of tuber shape must be on chromosome 7 and 10 respectively but unfortunately in our studies we did not find any association of marker at any chromosome. It is quite normal that some of the traits may remain without linked.

4.5.3.4 Tuber Size (TSz)

Tuber Size was analyzed after harvesting and normal distribution curve was observed over the range of readings that were taken. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, Quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale and following results were obtained.

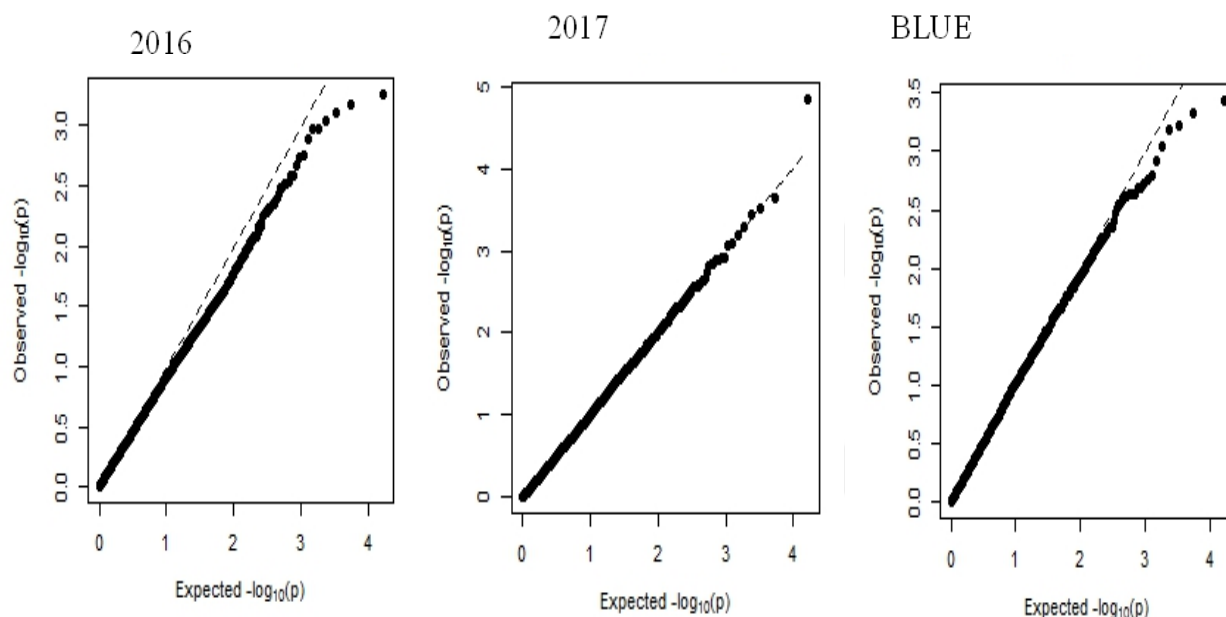


Figure 4.56. Q-Q plots for the trait of tuber size for the year 2016, 2017 and BLUE

We observed that for the data set of 2016, the observed values were below the expected p value line just after 1 and remained below the line until end. For the data set of 2017, we found an ideal graph right from the start to the end. For the data set of BLUE, up to 3 the observed values followed the expected values line quite well but after that they tend to get below the line and remained below until end. Due to difference in data sets for two years resulting qq plots obtained were also not ideal in case of data set for the year 2016 and showed some deviation. Manhattan plots were drawn with the help of p values that we obtained from the TASSEL. For the data of 2016 and 2017 there was no SNP marker that was found to be associated with the tuber size of our potato genotypes. However, there was an SNP associated with tuber size but that was among the unmapped markers so that was with use to us.

However, in BLUEs there was SNP on chromosome 1 that was found to be associated with tuber size of potato crop (Table 4.24).

Table 4.24. SNP found in BLUE data set from GLM for tuber size

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c1_13317	1	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	5268	2.77E-07

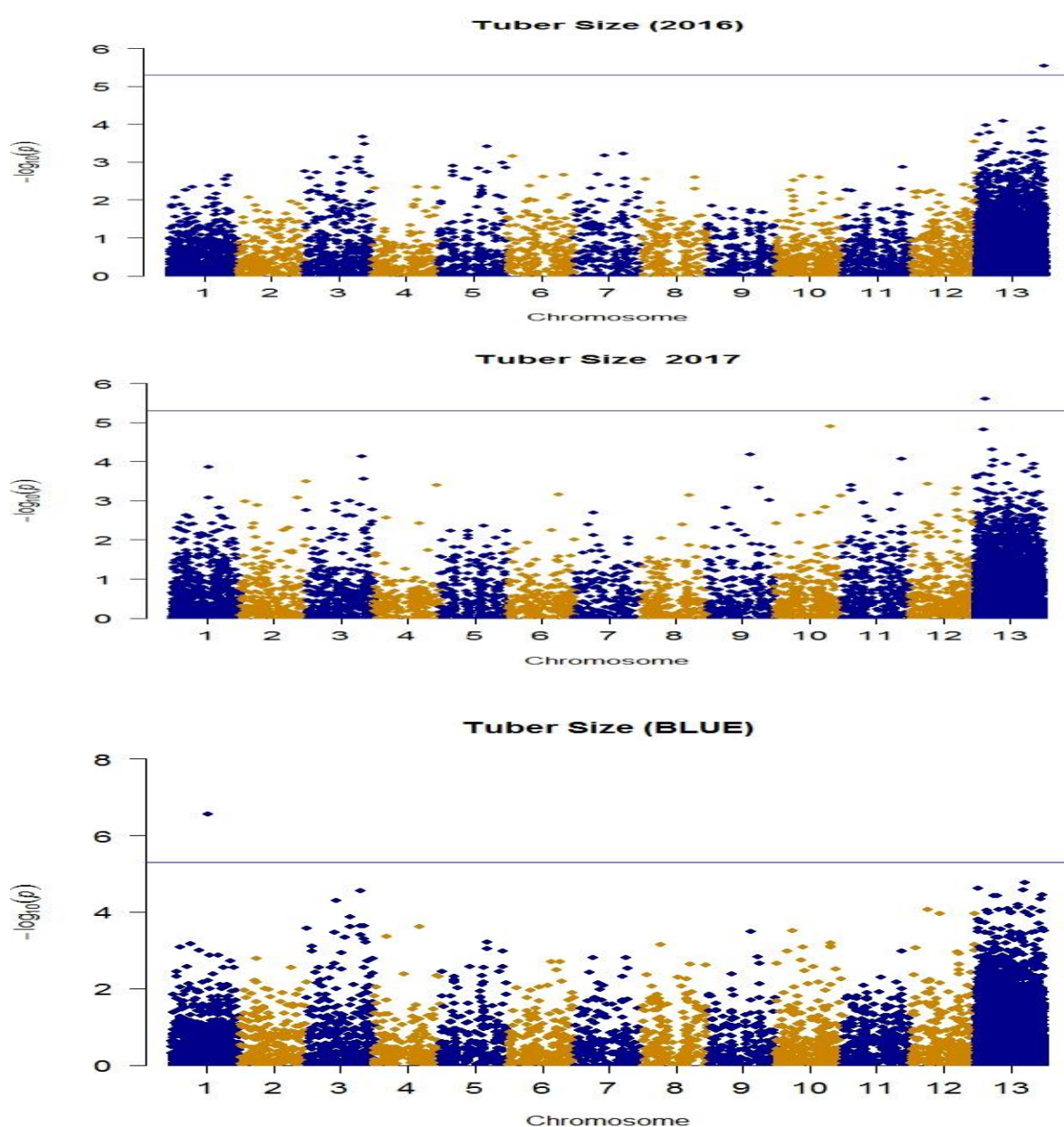


Figure 4.57. Manhattan plots using Generalized Linear Model for tuber size trait in potato

After analyzing the results with GLM, we also performed Mixed Linear Model (MLM) to our data and we found that in 2016 and 2017 data sets there was no SNP that was associated with the trait while for the case of BLUEs there was a single SNP on chromosome 1 (Table 4.25).

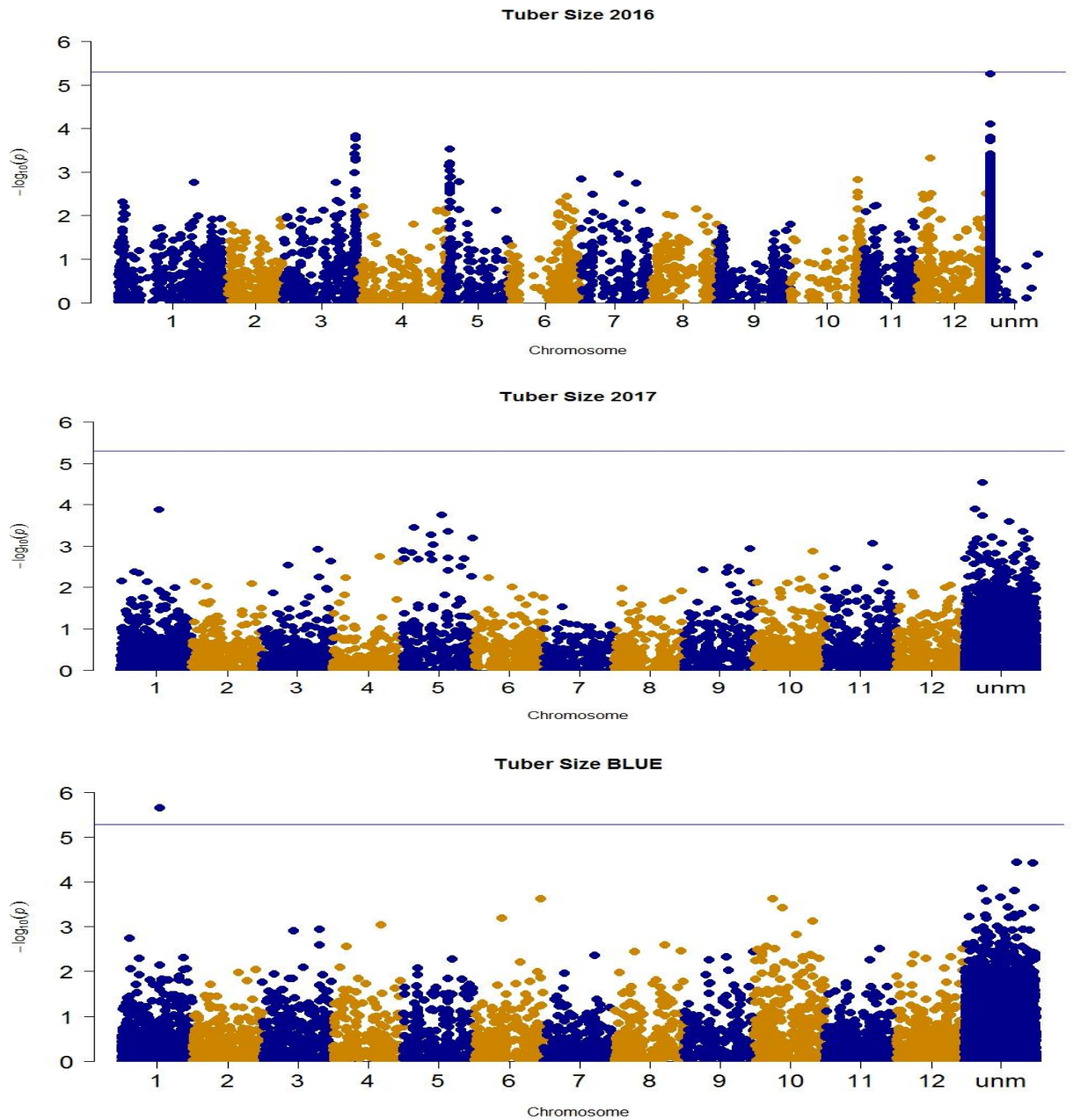


Figure 4.58. Manhattan plots using Mixed Linear Model for tuber size trait in potato

Table 4.25. SNP found in BLUE data set from MLM for tuber size

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c1_13317	1	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	5268	2.23E-06

Rak et al., 2017 while undergoing with their research on QTL mapping of potato chip color and tuber traits within an autotetraploid family using SNPs found that the trait of tuber size is linked on chromosome number 5. In our studies we found the association of SNP with this trait on chromosome number 1 of potato genome. This marker solcap_snp_c1_13317 on chromosome 1 can be considered for the future crop breeding programs or for marker assisted selection in potato for this trait.

4.5.3.5 Tuber Flesh Color (TFC)

A normal distribution curve was obtained for tuber flesh color. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale and following results were obtained.

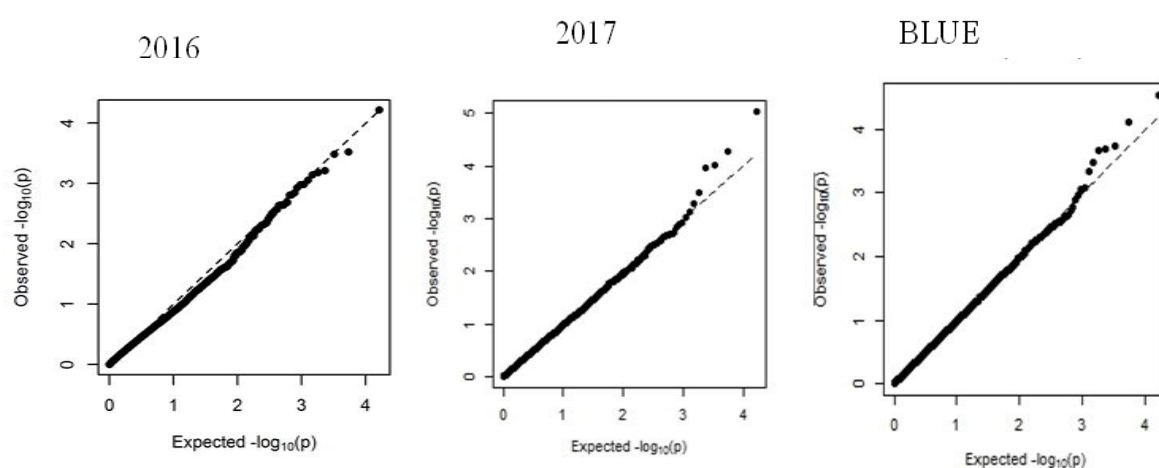


Figure 4.59. Q-Q plots for tuber flesh color of potato for the year 2016, 2017 and BLUE

For each data, ideal Q-Q plots was formed in case of tuber flesh color that means the observed valued followed the expected values fairly well and at the end for each data set they tend to move above a bit from the expected valued giving some clue for the positive associations among the trait and the SNPs.

Despite getting good Q-Q plots we found no single association among SNPs with tuber flesh color. Only in 2017 data there was a single marker that was found to be associated with tuber flesh color. But that was not any chromosome but that was among the markers that remained unmapped.

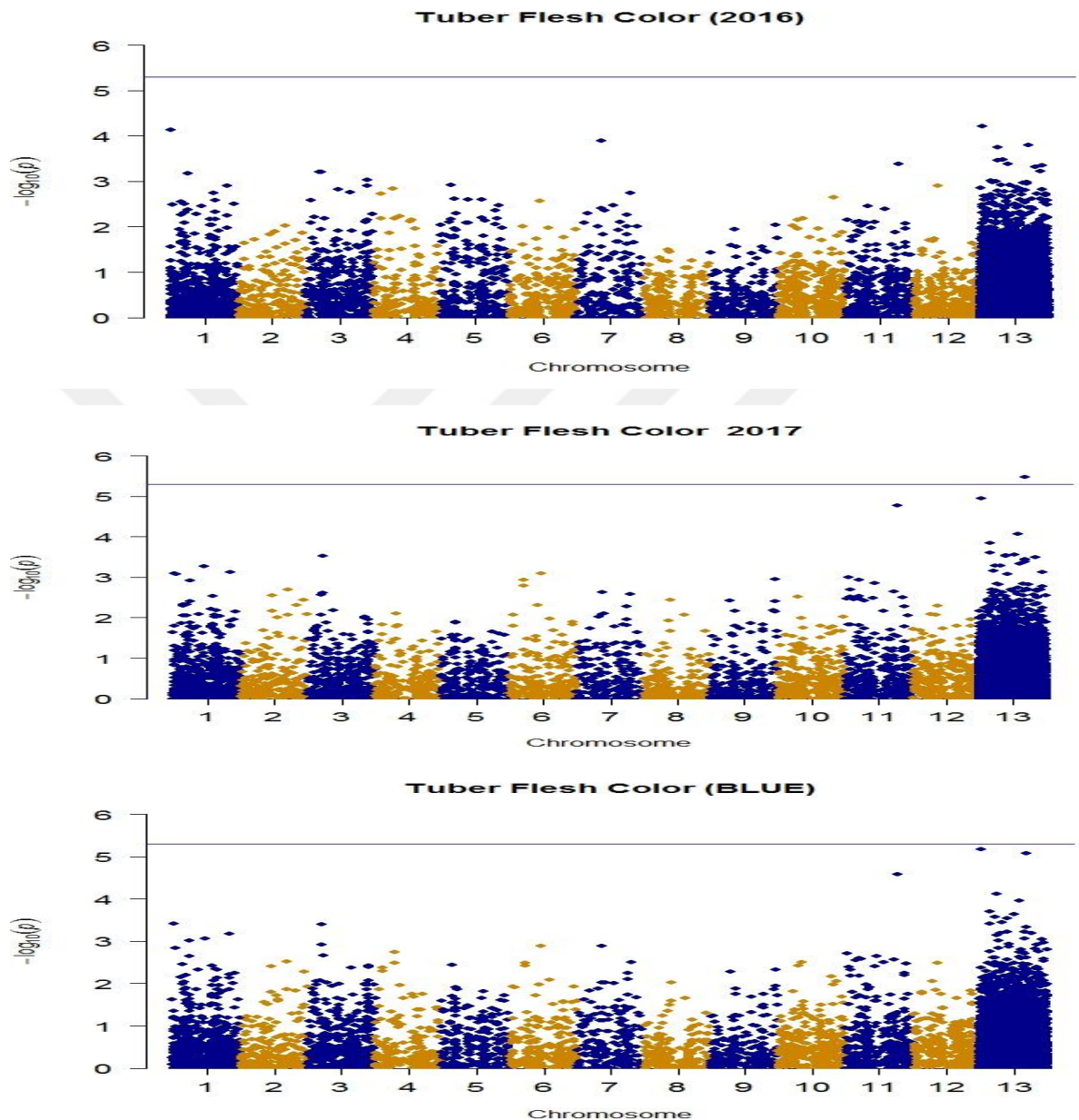


Figure 4.60. Manhattan plots using Generalized Linear Model for tuber flesh color trait in potato

After analyzing the results with GLM, we also performed Mixed Linear Model (MLM) to our data and there was no SNP associated to the tuber shape of the potato crop. All the SNPs were below the threshold level showing no association to the trait.

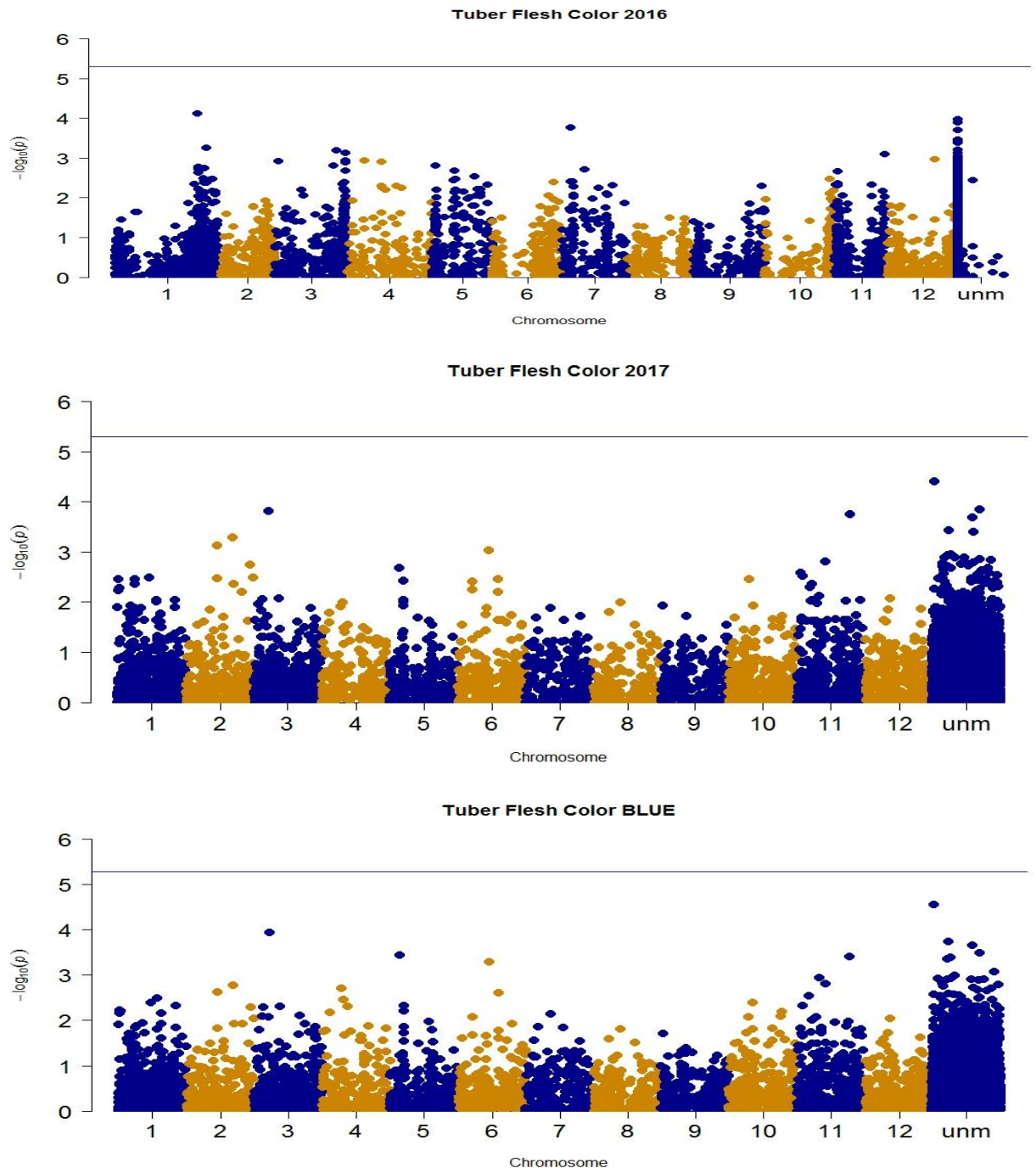


Figure 4.61. Manhattan plots using Mixed Linear Model for tuber flesh color trait in potato

These findings were not according to the findings of Berdugo-Cely et al., 2017 and Van Eck et al., 1994a, Bonierbale et al., 1988 and Jacobs et al., 1995 who reported that the trait of tuber flesh color must be on chromosome 7 and 3, 10 respectively but unfortunately in our studies we did not find any association of marker at any chromosome.

4.5.3.6 Tuber Skin Texture (TST)

A normal distribution curve was obtained for tuber flesh color. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale and following results were obtained except for the data set of year 2017, which tend to get below the line from 2 to 3.5 and started to get higher again after that and at the end the last point was found to be exactly on the expected line.

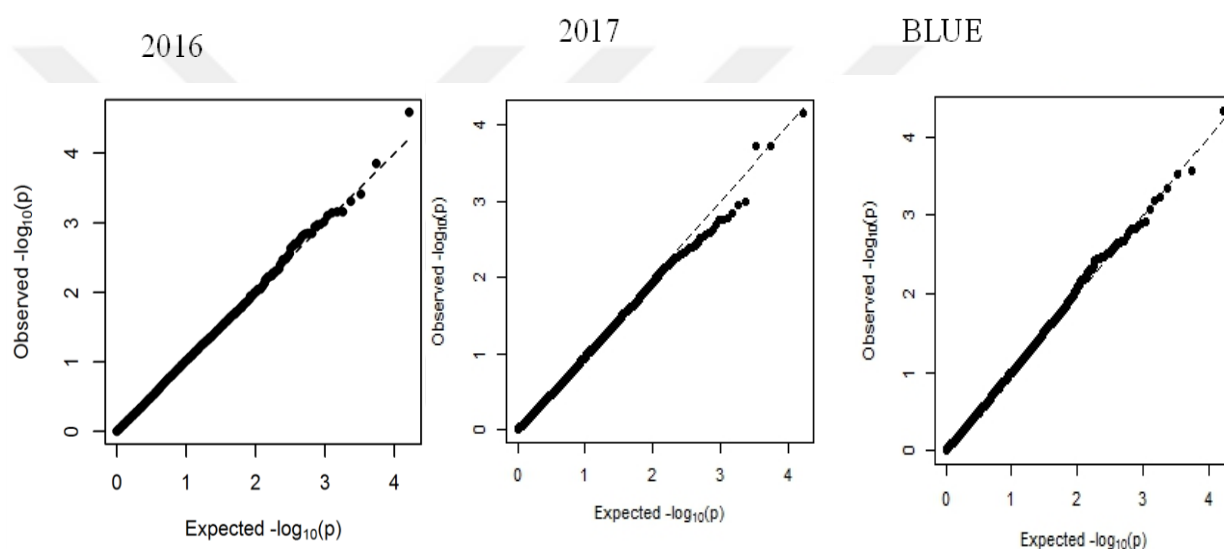


Figure 4.62. Q-Q plot for tuber skin texture of potato tuber for the year 2016, 2017 and BLUE

After Q-Q plots when Manhattan plots were drawn by using p values we plotted Manhattan plots and found no significant marker to be associated with tuber skin texture of the potato crop.

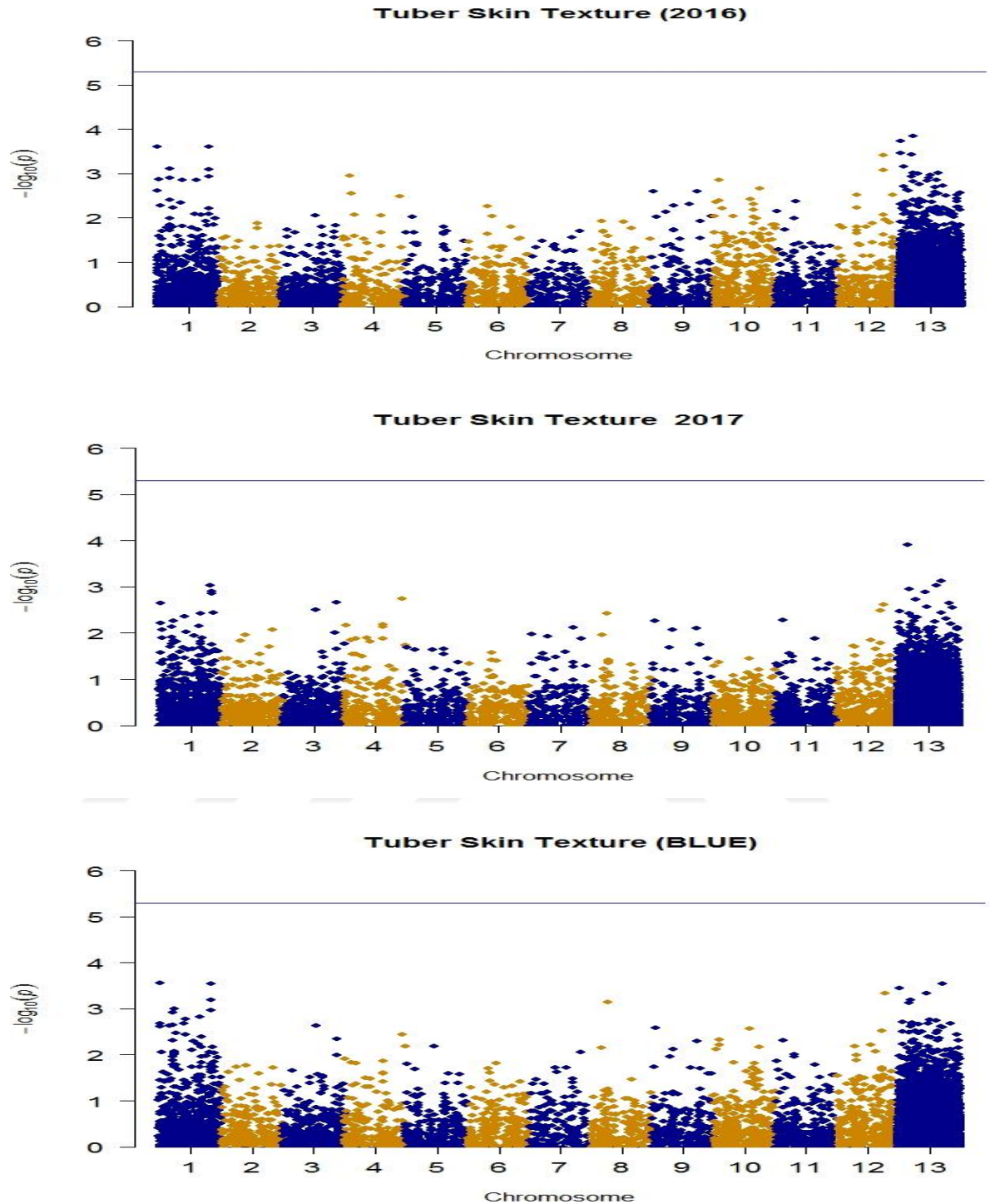


Figure 4.63. Manhattan plots using Generalized Linear Model for tuber skin texture trait in potato

After analyzing the results with GLM, we also performed Mixed Linear Model (MLM) to our data and there was no SNP associated to the tuber shape of the potato crop. All the SNPs were below the threshold level showing no association to the trait.

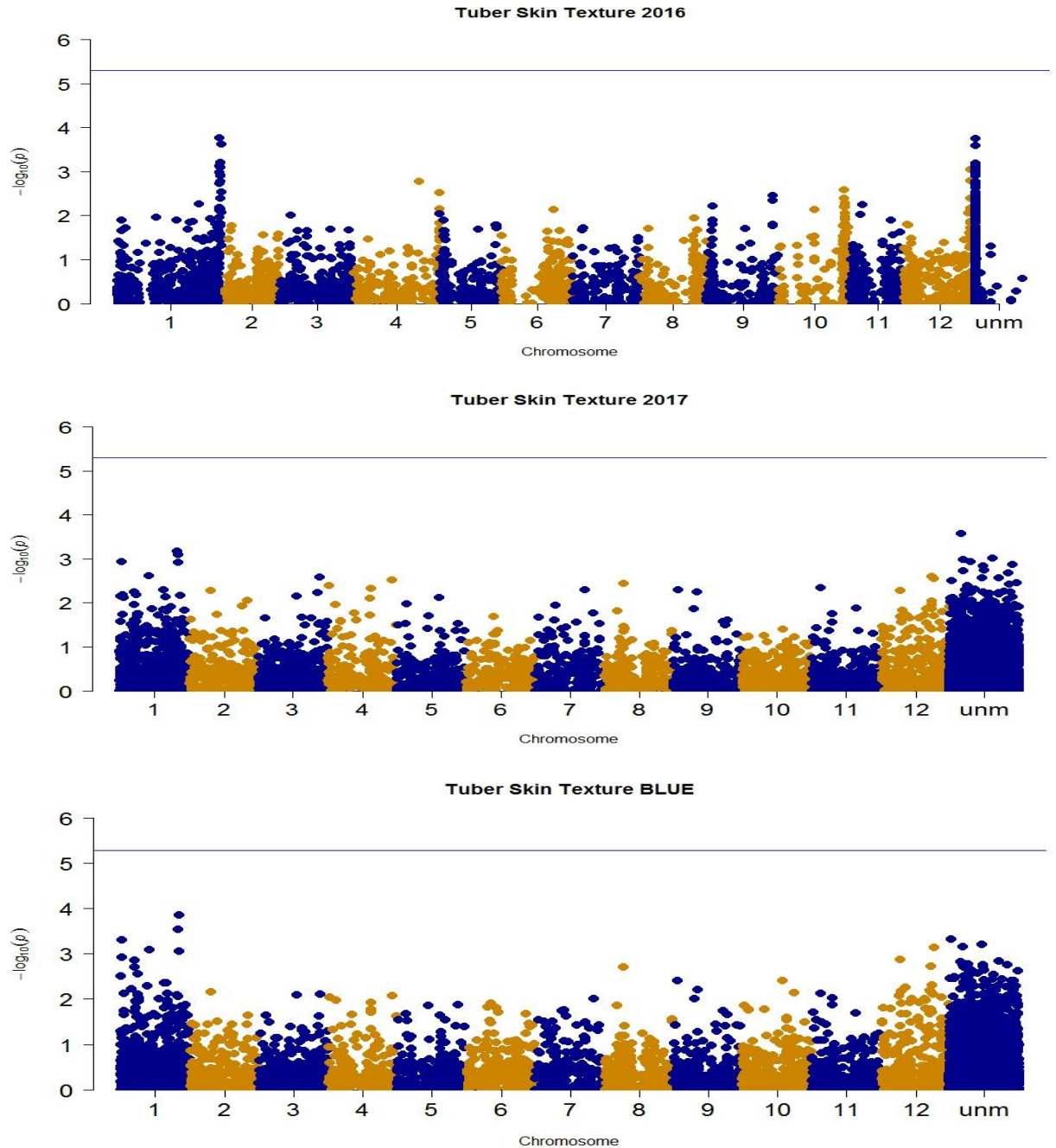


Figure 4.64. Manhattan plots using Mixed Linear Model for tuber skin texture trait in potato

McCord, 2009 explained for the first time in his studies about tuber skin texture using SSRs marker system. He observed the QTLs for potato skin texture on chromosome number 3 and 9 when he was using one of the potato lines named as B1829-5 and Atlantic.

4.5.3.7 Tuber Yield (TY)

A normal distribution curve was obtained for tuber flesh color. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale and we found that the data set 2017 and BLUE were found with ideal graphs but for the data set of year 2016, after 2.5 the observed values went under expected values up to the end.

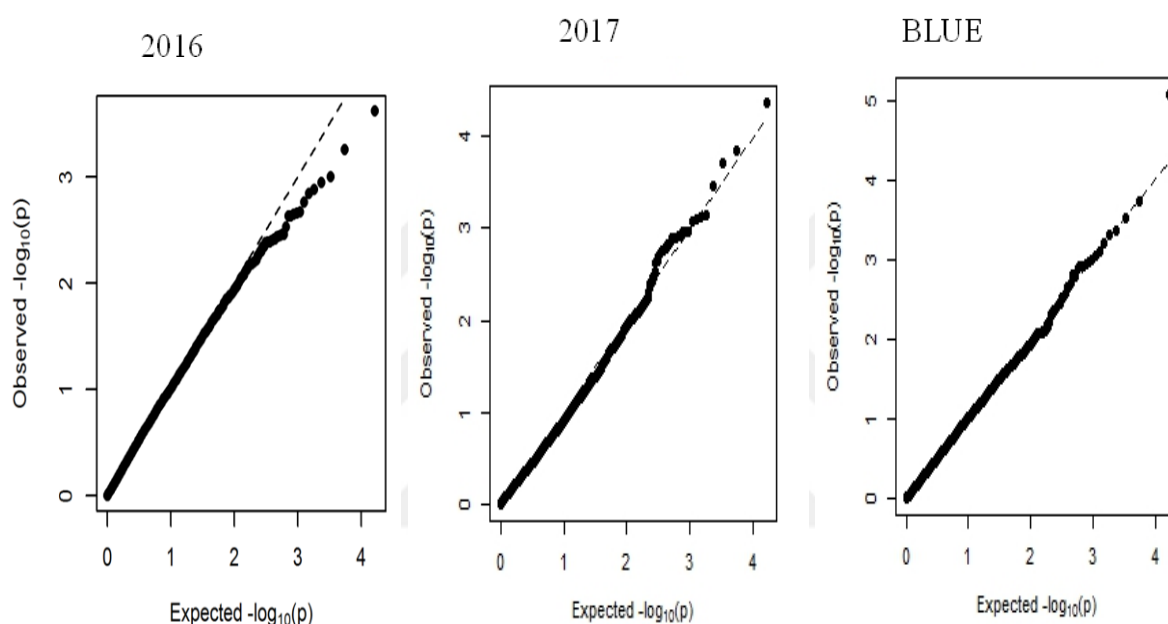


Figure 4.65. Q-Q plots for tuber yield of potato crop for the year 2016, 2017 and BLUE

Manhattan plots were drawn taking p values from TASSEL. We got the following results. For the year 2016 data we got five (05) SNP markers to be associated with tuber yield of potato crop 4 of them was among the unmapped markers and one associated SNP was on chromosome 3 (Table 4.26).

Table 4.26. SNP found in 2016 data set from GLM for tuber yield

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_46600	3	Short-chain dehydrogenase TIC 32	7960	6.22E-08

For the Manhattan plot from the year 2017 data we found no SNP to be associated with tuber yield of potatoes. But for BLUEs we got some associated markers that were distributed among the unmapped and on chromosome 3 and 5 (Table 4.27)

Table 4.27. SNP found in BLUE data set from GLM for tuber yield

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_46600	3	Short-chain dehydrogenase TIC 32	7960	1.31E-09
solcap_snp_c2_56223	3	Protein IQ-DOMAIN 14	4272	7.61E-08
solcap_snp_c1_7358	5	Beta-1,3- galactosyltransferase 15	9077	2.30E-06

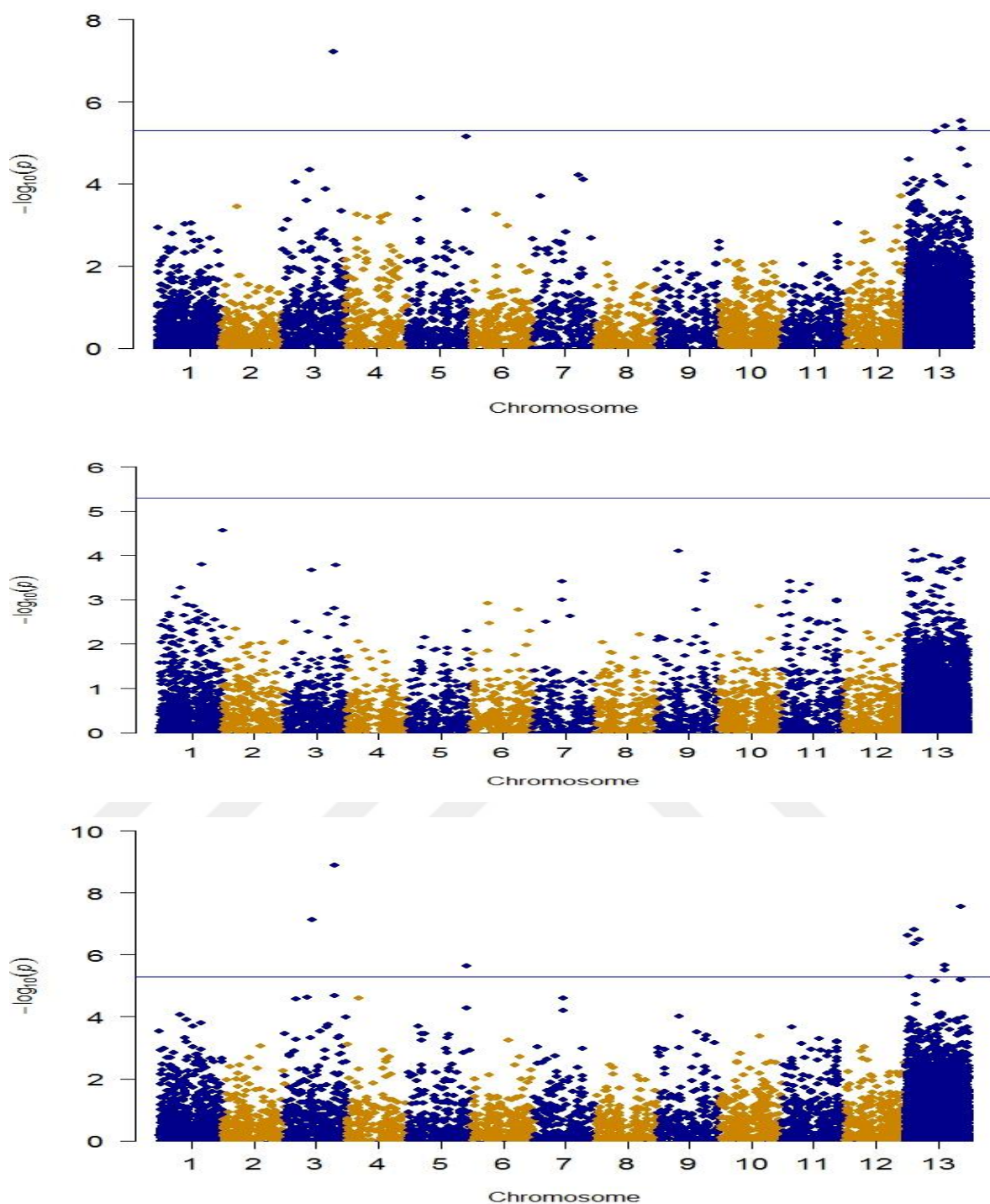


Figure 4.66. Manhattan plots using Generalized Linear Model for tuber yield trait in potato

Among 2016 and BLUE there was a common SNP `solcap_snp_c2_46600` that was located on chromosome 1. Mixed Linear Model (MLM) was performed to the data sets and no SNP was found to be associated with tuber yield trait of potato crop. In the data sets of year 2016 and 2017 there was no SNP even near to the threshold line. But in

BLUES, on chromosome 2 there was an SNP that was found to be very near to the threshold line but, yet it was not said to be associated with the trait.

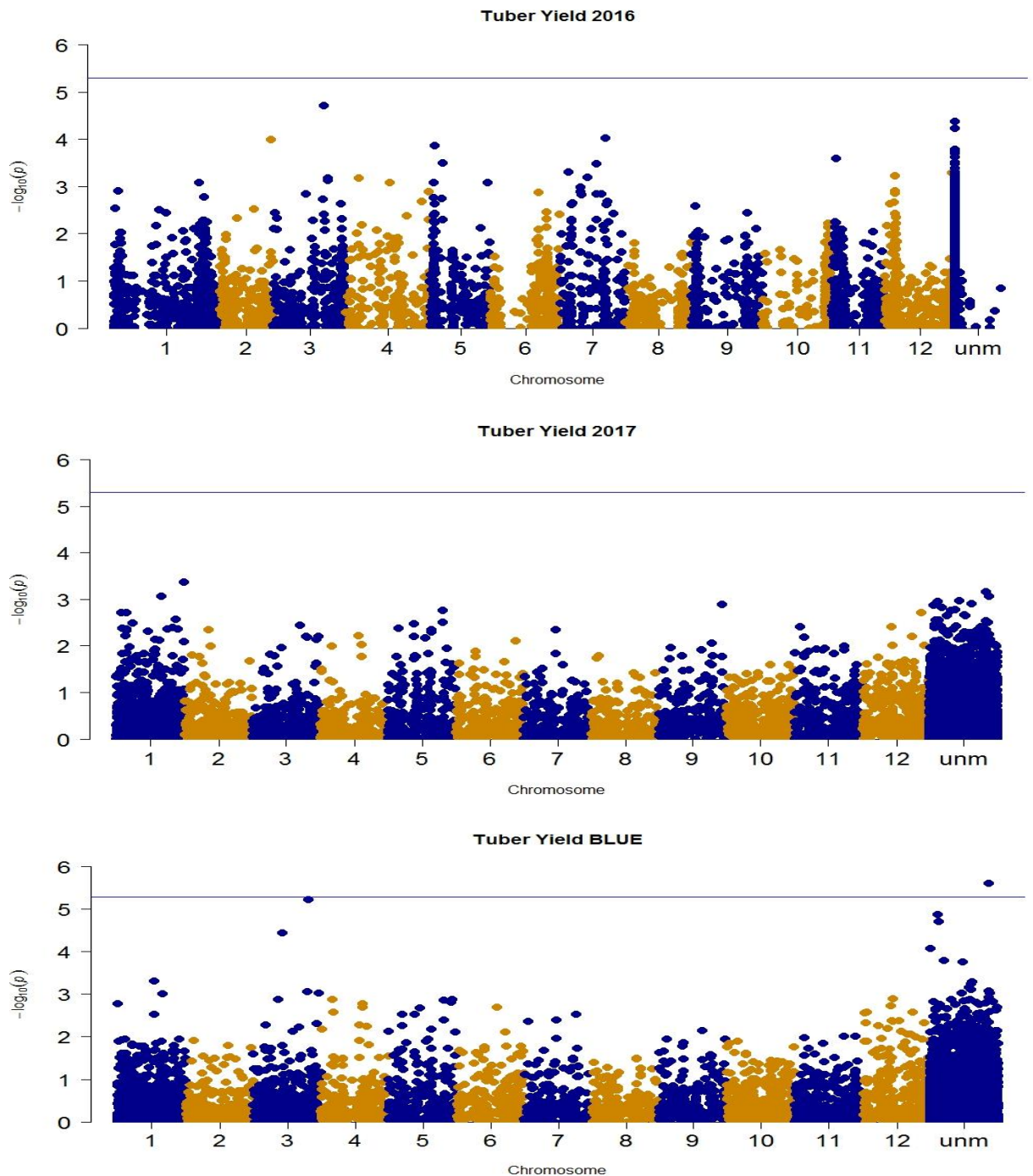


Figure 4.67. Manhattan plots using Mixed Linear Model for tuber yield trait in potato

These results are in accordance with the findings of Manrique-Carpintero et al., 2015 where they found that tuber yield is associated on chromosome number 5 along with chromosome 2 and 12. In our findings we found that the trait of tuber yield is associated

on chromosome number 5. Besides chromosome 5, from our GLM analysis, we found chromosome number 3 to be associated with tuber yield trait. We can consider the SNP present on chromosome 3 (solcap_snp_c2_46600) for our future studies as it was common in GLM as well as it was found to be close enough with the threshold level. So, this marker can be suitable for further studies specially on tuber yield trait of potato.

4.5.4 Tuber Quality Traits

4.5.4.1 Specific Gravity (SG)

A normal distribution curve was obtained for tuber flesh color. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples, quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale and following results were obtained.

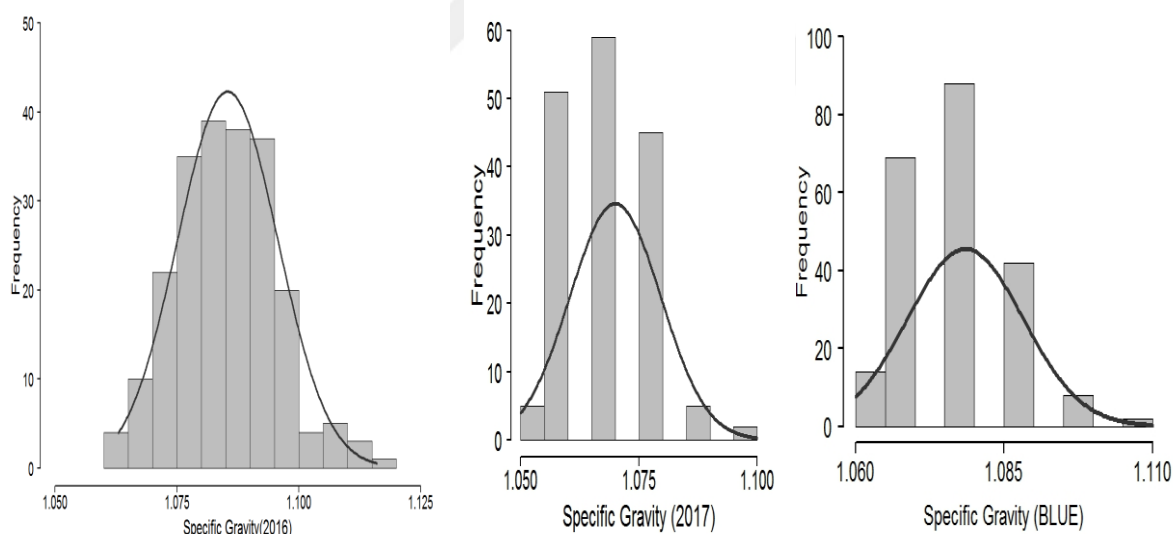


Figure 4.68. Bar plot for the trait specific gravity of potato crop for the year 2016, 2017 and BLUE

For the data of 2016, we got the perfect Q-Q plot following the same expected values. For the year 2017, the graph seemed to be bending at the ending showing that the p values is decreasing at the end. For BLUEs, initially the graph was according to the expected values but at the end it bended a bit and then again at the end it was exactly on the expected p value line.

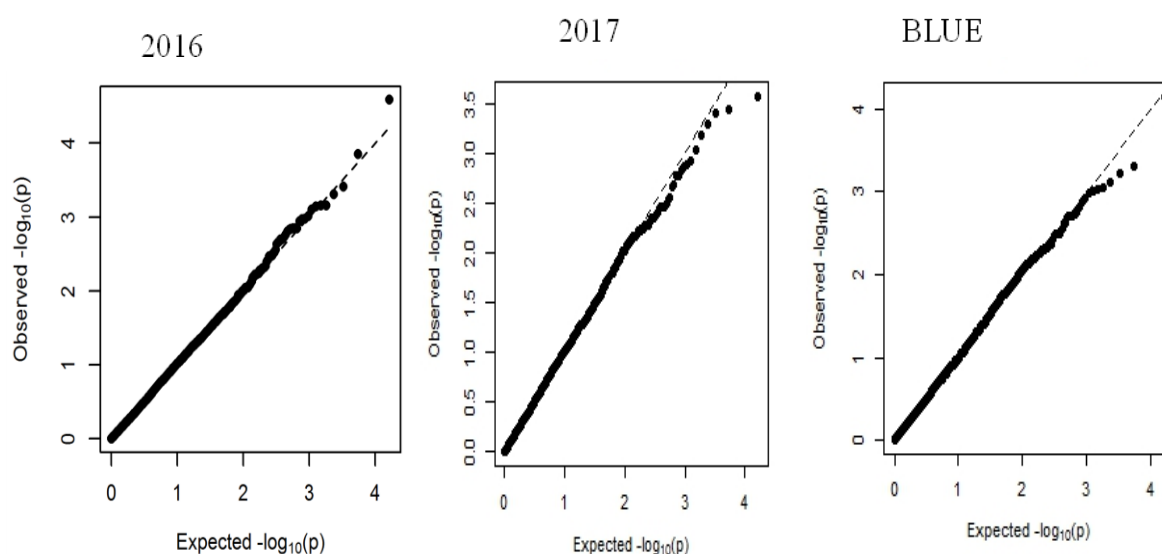


Figure 4.69. Q-Q plot for specific gravity of potato crop for the year 2016, 2017 and BLUE

Manhattan plots were drawn after Q-Q plots with the help of p values which were obtained from TASSEL. For the year 2016, we observed that beside unmapped markers there was a marker that was associated with specific gravity on chromosome 1 (Table 4.28)

Table 4.28. SNP found in 2016 data set from GLM for specific gravity

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_16033	1	Uncharacterized	2160	1.35E-07

For the data of 2017 again, the same pattern was found. One marker that was significantly associated with the trait was found among the unmapped markers and one of SNP marker was found on chromosome 1 (Table 4.29)

Table 4.29. SNP found in 2017 data set from GLM for specific gravity

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_48560	1	Uncharacterized	8064	4.31E-06

For the Manhattan plot that was from BLUEs we found no significant marker associated with the trait except the ones which were among unmapped markers.

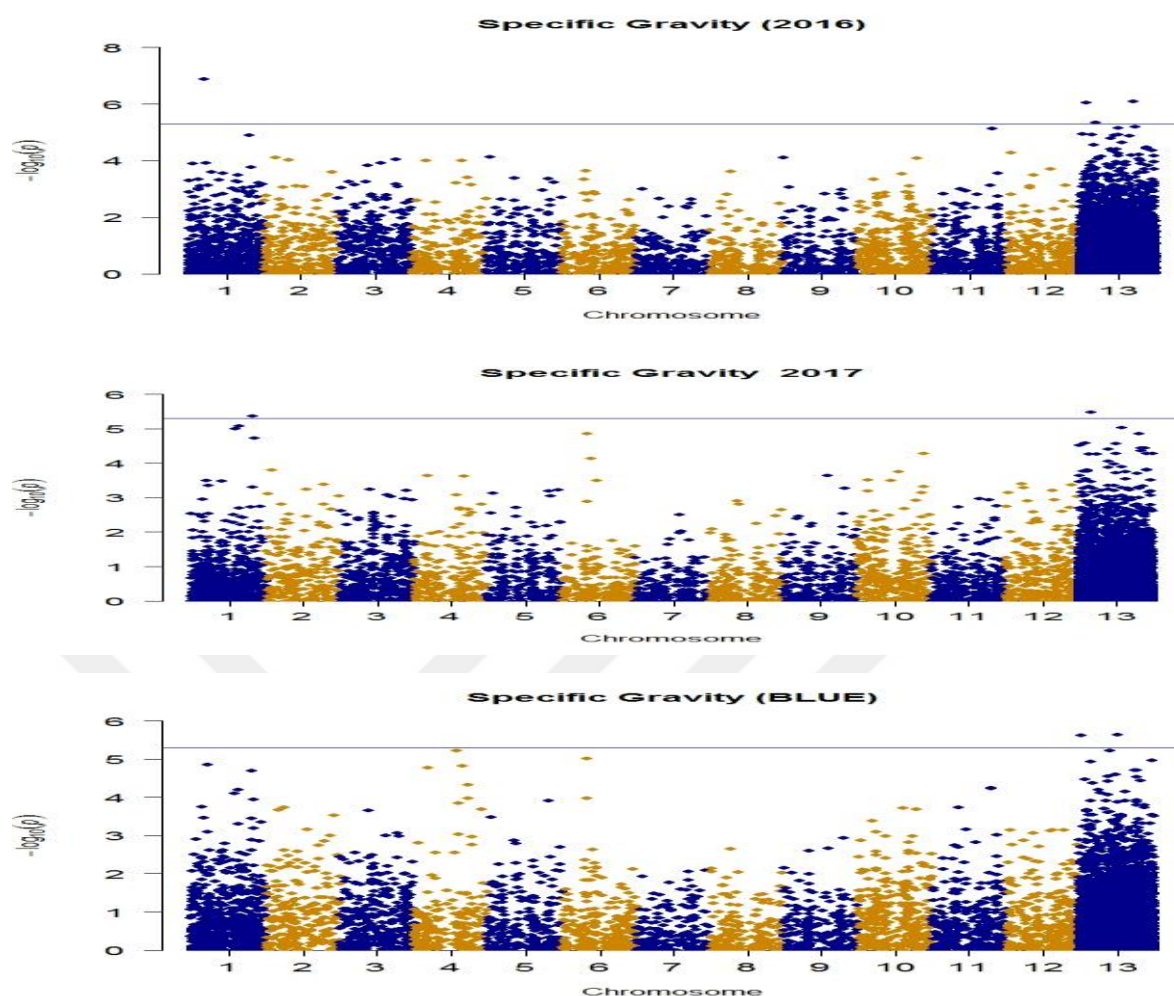


Figure 4.70. Manhattan plots using Generalized Linear Model for specific gravity in potato

MLM was performed also for specific gravity of potato tubers and found that in data sets of year 2016 and 2017 data there was some SNPs that were associated with Specific Gravity but for the Manhattan plots of BLUEs readings we found no SNP to be associated with the trait (Table 4.30).

Table 4.30. SNP found in 2016 data set from MLM for specific gravity

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_16033	1	Uncharacterized	66900423	9.41E-07

In the data of 2017, one SNP on chromosome 1 was found (Table 4.31).

Table 4.31. SNP found in 2016 data set from MLM for specific gravity

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_13414	1	polyol transporter 5	6211	4.75E-06

For BLUEs, there was no associated marker with specific gravity. Some SNPs on chromosome 4 were found to be near to the threshold line but they cannot be said to be associated with the specific gravity.

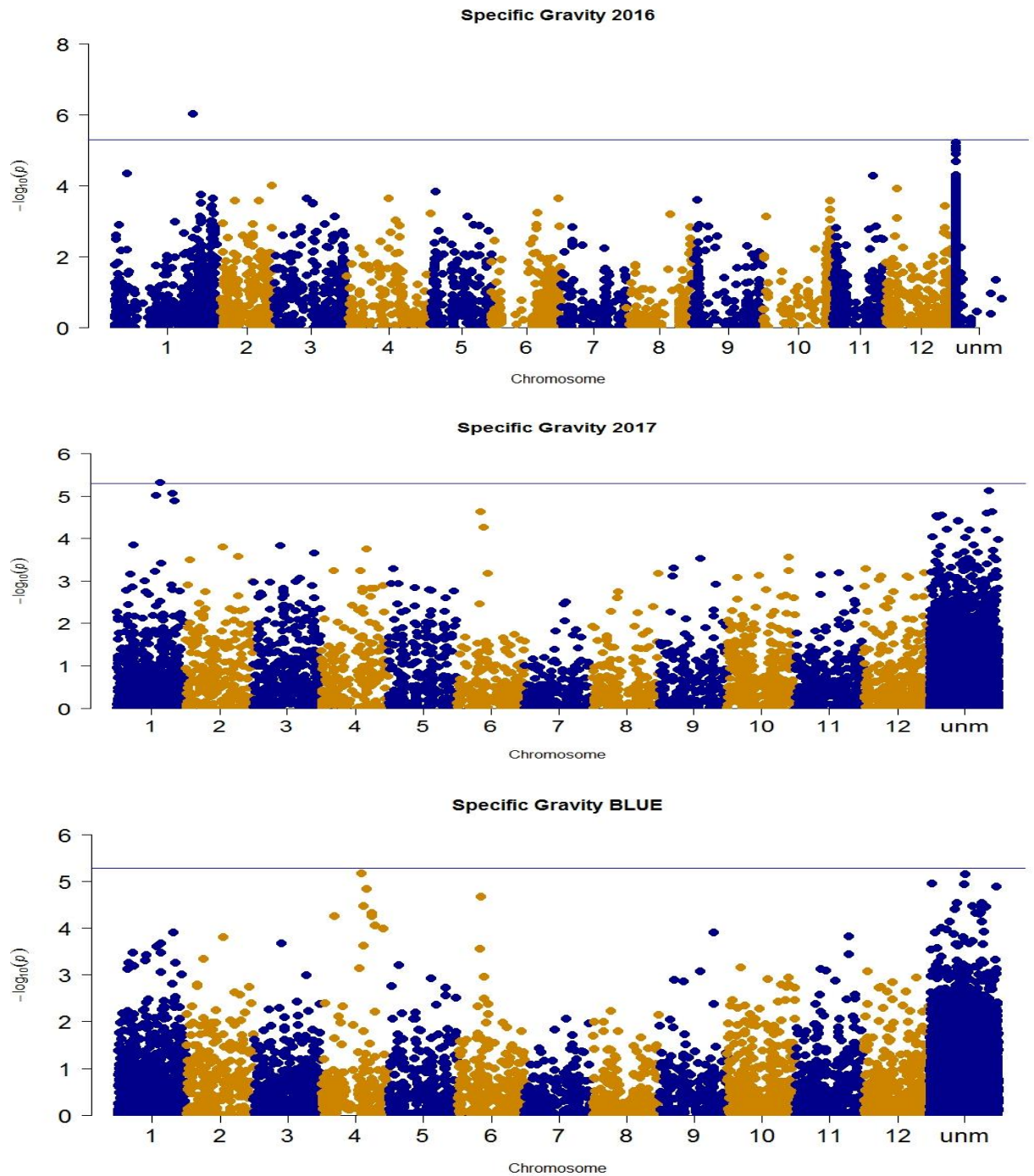


Figure 4.71. Manhattan plots using Mixed Linear Model for specific gravity in potato

These results are not according to the findings of Manrique-Carpintero et al., 2015 where they described after concluding the results from QTL analysis that specific gravity is associated on chromosome number 5 and 9 but for our results we found the association on chromosome number 1 for the trait of specific gravity. We can consider solcap_snp_c2_16033 marker on chromosome 1 for our further studies for specific gravity of potato.

4.5.4.2 Dry Matter Content (DMC)

A normal distribution curve was obtained for tuber flesh color. For validating either the model that we are practicing is appropriate for the control of population stratification, family structure as well as the false positives among the samples,

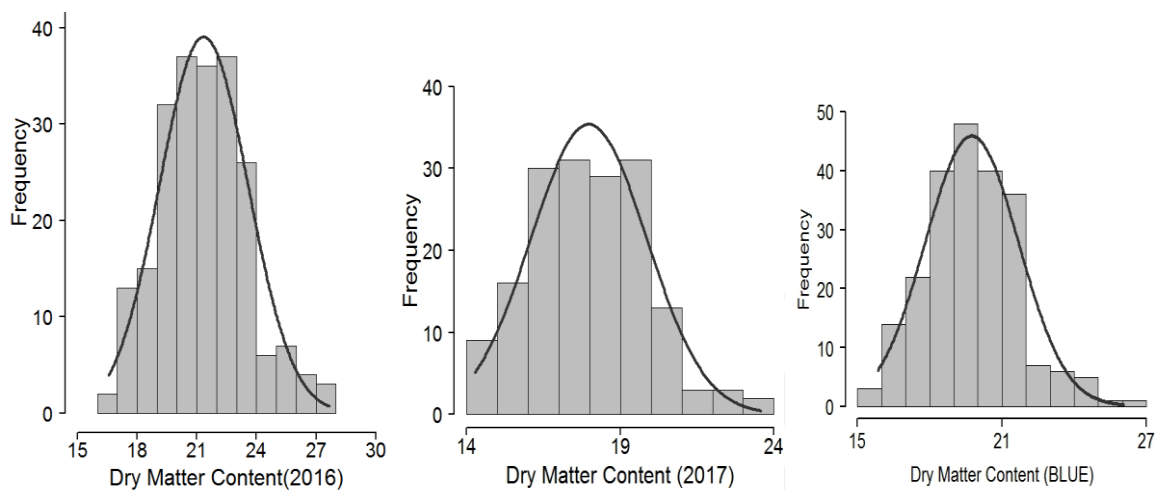


Figure 4.72. Bar plots for dry matter content of potato crop for the year 2016, 2017 and BLUE

Quantile-quantile plot depicting expected versus observed P values at $-\log_{10}$ scale and following results were obtained. Q-Q plots for each data set were found to be bending at the end of the plot. Except the end where the readings were found to be as expected p values.

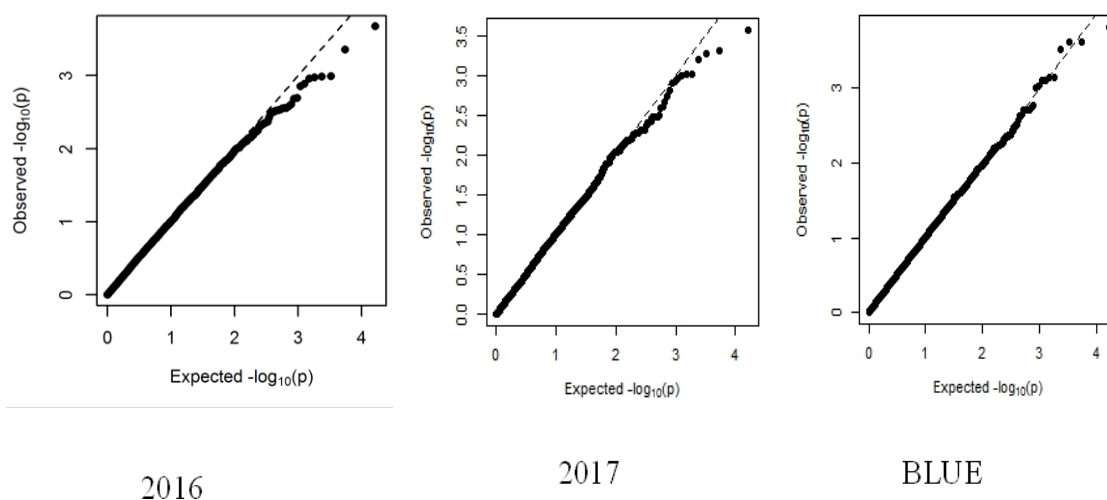


Figure 4.73. Q-Q plot for dry matter content of potato crop for the year 2016, 2017 and BLUE

Manhattan plots were drawn with p values that we obtained from TASSEL. We found that for the Manhattan plot for the year 2016, there was a significantly associated SNP on chromosome 1. Another SNP was also found to be associated with Dry Matter Content but that was among the unmapped markers (Table 4.32).

Table 4.32. SNP found in 2016 data set from GLM for dry matter content

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_16033	1	Uncharacterized	2160	8.70E-09

For the Manhattan plot that we obtained from the data set of 2017, we found no SNP to be associated with dry matter content of potato crop.

For the Manhattan plot that we obtained from BLUEs, we found that there were around 6 SNP markers that were associated with dry matter content of potato crop. 4 of them was among the unmapped markers and the other two were on chromosome 1 and 6 respectively (Table 4.33).

Table 4.33. SNP found in BLUE data set from GLM for dry matter content

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_16033	1	Uncharacterized	2160	2.18E-07
solcap_snp_c2_41237	6	nudC domain-containing protein 2	3414	4.26E-06

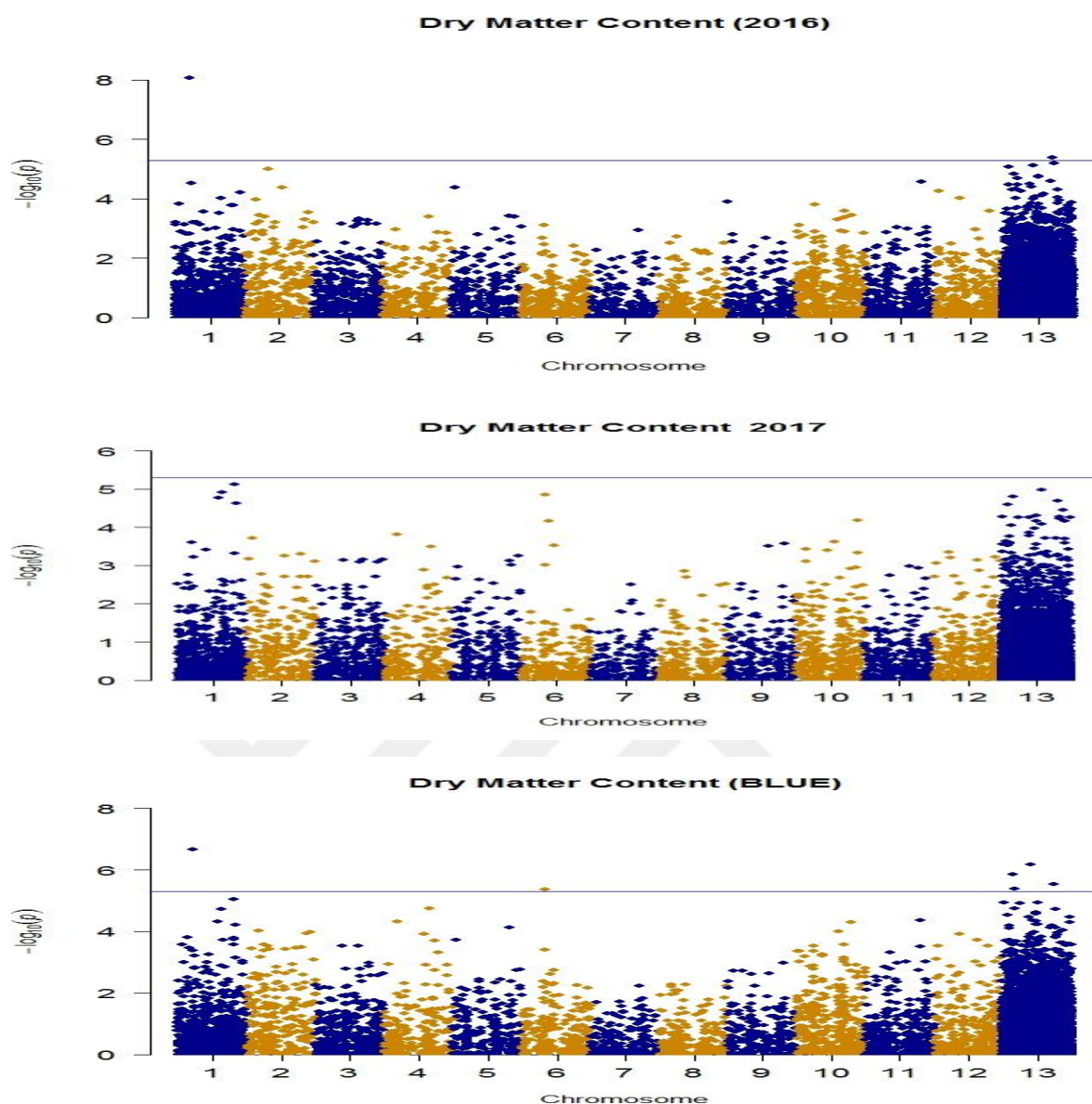


Figure 4.74. Manhattan plots using Generalized Linear Model for dry matter content of potato

We found that for the data set of 2016 and BLUEs, there was a common SNP `solcap_snp_c2_16033` that was associated with dry matter content of potatoes.

After GLM mixed linear model (MLM) was also performed and found the results like, For the data of 2016, there was no SNP found to be associated with dry matter content of potato tubers.

For the data of year 2017, on chromosome 1 there was a single SNP found to be associated with dry matter content (Table 4.34).

Table 4.34. SNP found in 2016 and 2017 data set from MLM for dry matter content

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_13414	1	polyol transporter 5	6211	5.38E-06

For the Manhattan plots that came from BLUEs, there was a single SNP on chromosome 6 besides the unmapped markers that was found to be associated with dry matter content (Table 4.35).

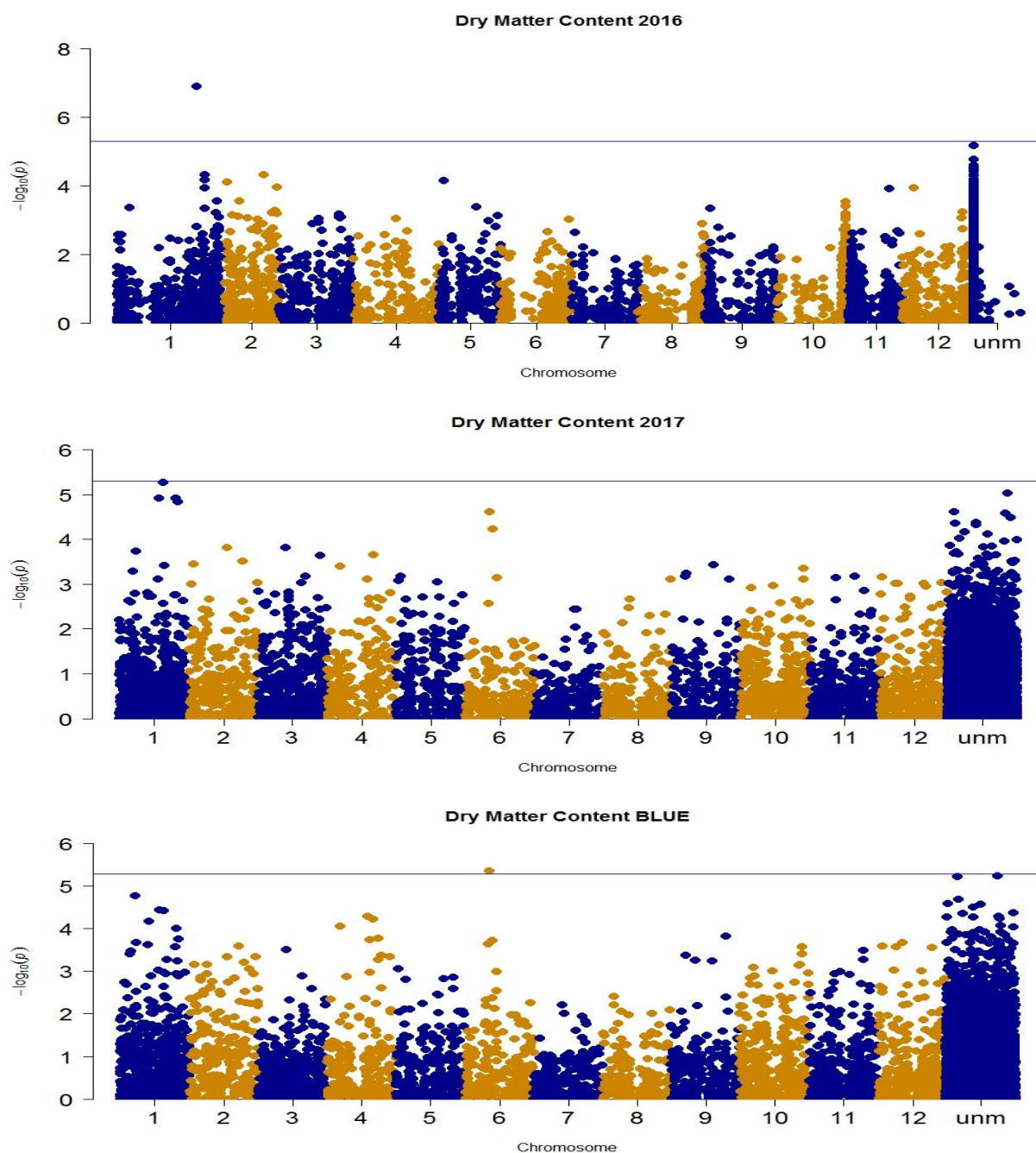


Figure 4.75. Manhattan plots using Mixed Linear Model for dry matter content of potato

Table 4.35. SNP found in BLUE data set from MLM for dry matter content

SNP	Chromosome	Putative Function	Base pair	P Value
solcap_snp_c2_41237	6	nudC domain-containing protein 2	3414	4.47E-06

For Dry Matter Content (DMC), it was observed that in both GLM and MLM for the data set of BLUEs, solcap_snp_c2_41237 was the SNP that was found common in both the models.

These results are not in accordance with the results of McCord et al., 2011 where they presented by using their studies on QTL analysis on potato and described that dry matter content of potato is linked or associated on the chromosome 2,5,8,9 and 11. But for our studies we found dry matter content is associated on chromosome number 1 and 6. Solcap_snp_c2_16033 marker on chromosome 01 and solcap_snp_c2_41237 marker on chromosome 06 can be considered for next crop breeding programs or as a marker assisted selection of potato.

It is quite normal that the data in one year can be changed for the next year due to different reasons like genotype and environment interaction that causes the change in functions of the alleles and also the behavioral changes can also be found due to this interaction. To get more reliable data, phenotyping should be repeated for the number of years keeping more genotypes under study. Berdugo-Cely et al., 2017 repeated their experiment for 8 different years keeping 809 genotypes and for three different locations. So, to be sure and to get some reliable results, phenotyping should be repeated as many times as it could be. Increased number of genotypes, years, locations and environment effect the association mapping results. The markers found in association studies should be validated first before using them as candidate markers for future breeding programs. Most of the straightforward way to prove a marker trait association is to evaluate the candidate polymorphism in an entirely different and independent population sample. A potential failure of validation may be the result of gene and environment interaction or gene into genetic background. Further reasons of failure could be a low power to detect DNA marker and phenotype association in a population in which validation is being practiced. Second reason could be the spurious marker trait associations in the initial population caused by statistical over interpretation of results. We can also use different

molecular techniques to validate the markers. Kompetitive Allele Specific PCR (KASP), Semi-Thermal Asymmetric Reverse PCR (STARP) as well as Cleaved Amplified Polymorphic Sequences (CAPS) could be used to validate the candidate markers. KASP and STARP are the techniques that use florescent probes that use qPCR to proceed. CAPS works on the basis of restriction enzymes so these can be used by using simple PCR. The cut bands can be seen by running simple gel electrophoresis technique. Additionally, conventional potato plant breeding programs could be supported using the genetic information through marker-assisted selection (MAS) and genomic selection (GS), and thus MAS and GS accelerate the selection of potato materials and reduce the cost and time to develop new potato varieties.

Hence, the SNPs found can be very useful in terms of future studies and can provide a beam of light for many scientists to think about this aspect as one of the important goal of plant breeding is to get the elite line. As compared to the traditional selection approach that is basically based on phenotypes. Selecting the genotypes based on genomes and by using these kinds of SNPs would be very helpful for accelerating the speed of breeding program in a less expensive and more efficient way. The results from our studies explain that genome selection can attain increased level of accuracy for the prediction of traits and justifying those traits in the crop breeding program.

CHAPTER V

CONCLUSION

Genome-wide Association (GWA) mapping technology has become the powerful tool to dissect the marker trait association in humans as well as in plants. With the study presented here in this thesis, a promising marker-trait association has been found for different plant traits of potato.

Plant Traits

- Solcap_snp_c2_16530 marker on chromosome 1 was found mainly to be associated with the trait of Growth Habit.
- For the trait branching pattern three different SNPs (solcap_snp_c2_9611, solcap_snp_c2_9557 and solcap_snp_c1_131) on chromosome 3 were found to be associated.
- For Plant Height different SNPs from chromosomes 1, 3, 5, 6 and 11 were found to be associated but mainly SNPs (solcap_snp_c2_55972, solcap_snp_c2_47386, solcap_snp_c2_57886, solcap_snp_c2_39960) on chromosome 11 was found to be associated with plant height.
- In case of Stem Thickness, solcap_snp_c2_4274 on chromosome 11 was found to be associated.
- For the traits like Number of leaflets per plant and leaf shape we were unable to find any associated marker associated with the specific trait.
- For maturity of the plants different SNP markers were found to be associated on chromosomes 1, 2, 3, 11, 12.

Flower Traits

- Solcap_snp_c1_13354 on chromosome number 4 was found to be associated with the Flower Color trait.
- Stamen length was found to be associated with 06 different SNPs present on chromosome number 3, 5, 6 and 7.
- For Pistil length and Pistil length above stamen we were unable to find any associated marker on any of the chromosome.

Tuber Traits

- Tuber skin color was found to be associated with 06 different markers present on different chromosomes. But mainly chromosome number 3 with solcap_snp_c2_244, solcap_snp_c2_247 and solcap_snp_c2_250 markers.
- Solcap_snp_c2_29272 on chromosome number 7 and solcap_snp_c2_36675 and solcap_snp_c2_6965 on chromosome number 1 was found to be associated with the trait of number of tuber per plant.
- We were unable to find any associated marker for the trait of Tuber Shape. Tuber Size was found to be associated with solcap_snp_c1_13317 marker on chromosome number 1.
- There was no marker that was found to be associated with Tuber Flesh Color and tuber skin texture. Tuber yield was found to be associated with solcap_snp_c2_46600 marker present on chromosome number 3 of potato.

Tuber Quality Traits

- Solcap_snp_c2_16033 and solcap_snp_c2_48560 were found to be associated with specific gravity of potato plant.
- Dry matter content was found to be associated with solcap_snp_c2_16033 on chromosome number 1 as well as solcap_snp_c2_41237 marker of chromosome number 6.

Data for one year can be different in next year due to different reasons that may include genotype and environment interaction that causes the change in functions of the alleles as well as allelic functions can be changed due to the interaction. To obtain more reliable data from association mapping, phenotypic data in the experiment should be repeated for a number of years. The number of genotypes in association mapping is also of great importance. In previous experiment, while working with association mapping in Colombian potato cultivars, repeated their experiment for 8 years with 809 genotypes and for three different locations. So, repeated and accurate phenotyping to get more reliable results is the key to successful and reliable results out of association mapping studies. Increased number of genotypes, years, locations and environment affect association mapping results. The results obtained should be validated before using them as a candidate markers and incorporating those in MAS or GS studies.

These results show number of markers associated with agronomic and morphological traits of potato which can be useful for the future studies designed on the basis of these markers or traits specially for developing the marker assisted breeding program in potato.



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APPENDIX-A Phenotypic data of nine (9) traits for the year 2016

Genotypes	Lpist	PLAS	Lsta	Maturity	NoLflts	PH	ST	ATS	BP
GENO1	8.588	2.435	6.09	2	26	50.127	0.764	2	2
GENO2	8.043	2.036	6.069	1	24	55.58	1.248	2	1
GENO3	7.932	1.888	6.219	2	20	40.971	0.837	2	2
GENO4	6.431	2.034	6.232	2	21	53.497	1.127	1	1
GENO6	9.611	2.483	6.937	2	15	59.588	0.814	1	2
GENO7	7.668	1.772	5.86	2	10	59.627	1.231	1	2
GENO8	7.964	2.213	5.768	1	35	49.597	1.139	1	1
GENO9	8.387	2.883	5.588	2	14	52.747	1.078	1	1
GENO10	8.744	1.252	7.611	2	33	42.019	0.86	1	2
GENO11	7.997	0.958	7.029	4	21	34.219	1.061	2	1
GENO12	9.326	2.647	6.841	2	27	39.099	1.024	1	1
GENO13	9.738	2.571	7.183	4	35	42.095	1.08	3	1
GENO14	7.399	1.632	5.757	2	20	38.919	0.992	1	1
GENO15	8.803	2.634	6.159	3	30	48.919	0.993	1	1
GENO16	8.497	1.88	6.607	4	20	38.919	0.892	2	1
GENO17	9.318	2.231	7.013	2	18	38.299	1.053	1	1
GENO18	9.224	2.202	6.989	4	30	42.971	0.961	2	1
GENO19	9.052	1.77	7.267	2	28	32.771	0.983	2	1
GENO20	8.749	1.478	7.323	3	33	49.471	0.883	1	2
GENO21	8.952	0.968	7.969	3	22	33.671	0.877	NA	2
GENO22	9.167	2.063	7.107	4	33	42.019	1.026	1	1
GENO23	8.319	1.389	6.619	3	27	51.591	1.026	2	2
GENO24	8.914	3.08	5.906	3	24	40.547	0.912	1	1
GENO25	9.24	1.734	7.677	3	22	26.28	1.011	1	1
GENO26	8.526	2.25	6.401	2	34	41.419	1.006	1	1
GENO27	10.2	2.969	7.245	2	38	41.299	0.929	1	1
GENO28	8.339	1.602	6.776	2	20	37.647	0.892	1	2
GENO29	8.045	2.308	6.014	4	20	37.968	1.009	1	1
GENO30	9.827	1.377	8.397	2	23	23.491	0.907	NA	1
GENO31	8.485	2.869	6.541	2	36	30.991	1.133	1	1
GENO32	7.924	1.52	6.388	3	19	38.647	0.946	1	1
GENO33	6.685	1.792	6.694	2	22	50.997	1.095	2	1
GENO34	9.879	2.44	7.429	2	34	50.919	0.816	1	2
GENO35	8.009	3.564	6.168	2	25	50.347	0.738	1	2
GENO36	7.992	2.095	5.828	2	22	36.068	0.827	1	2
GENO37	9.021	1.926	7.12	3	34	39.997	1.128	2	1
GENO38	8.454	1.768	6.473	2	25	46.088	1.085	2	1
GENO39	8.863	1.469	7.573	2	19	36.48	1.154	2	2
GENO40	8.997	1.738	7.366	3	27	34.468	1.157	2	1

APPENDIX-A (Continue) Phenotypic data of nine (9) traits for the year 2016

Genotypes	Lpist	LSAA	Lsta	Maturity	NoLflts	PH	ST	ATS	BP
GENO41	9.155	2.104	7.041	2	22	39.819	1.128	2	1
GENO42	10.074	1.137	8.279	3	19	26.391	0.855	2	2
GENO43	7.205	1.285	6.688	2	27	33.768	0.919	2	1
GENO44	10.473	3.404	7.103	3	29	42.58	1.176	2	1
GENO45	8.33	1.412	7.043	2	33	54.719	0.919	1	1
GENO46	6.99	2.031	7.05	2	14	28.768	0.908	NA	1
GENO47	9.599	1.257	8.328	2	25	40.547	0.914	2	2
GENO48	9.364	2.526	6.823	2	39	44.971	1.125	2	1
GENO49	8.262	0.92	7.398	2	31	43.747	1.063	2	2
GENO50	6.488	1.018	7.19	2	22	43.968	1.024	2	1
GENO51	6.717	2.092	6.082	2	39	50.747	0.973	1	2
GENO52	9.164	2.00	7.142	2	28	51.547	0.993	2	2
GENO53	7.103	1.304	7.624	2	20	41.897	1.076	2	1
GENO54	10.399	2.104	8.285	2	20	28.019	1.043	2	1
GENO55	8.059	1.89	6.159	4	28	37.419	1.13	2	1
GENO56	7.451	1.205	6.285	2	20	45.68	0.996	NA	2
GENO57	8.518	2.306	6.197	2	18	42.771	1.122	3	2
GENO58	8.678	2.964	5.747	2	24	50.095	0.996	1	1
GENO59	9.376	3.271	6.121	2	25	46.995	1.086	2	1
GENO60	7.738	2.631	7.102	2	26	44.368	1.056	1	2
GENO61	8.358	1.799	6.561	3	24	37.38	0.963	2	1
GENO62	7.467	2.43	4.929	5	14	48.971	1.007	1	1
GENO63	9.116	2.392	6.708	4	21	53.327	0.861	2	1
GENO64	8.216	2.05	6.291	2	43	35.819	0.996	1	2
GENO65	8.61	1.181	7.643	2	37	49.799	1.161	1	1
GENO66	7.309	0.875	6.692	2	18	37.647	1.18	1	1
GENO67	10.698	3.115	7.395	2	38	40.999	0.944	1	1
GENO68	10.324	2.652	7.737	2	29	30.619	0.68	1	2
GENO70	9.934	2.794	7.265	2	35	50.519	1.075	1	1
GENO71	8.338	2.648	5.815	2	18	35.019	0.841	1	1
GENO72	10.365	2.954	7.401	3	20	52.519	1.326	NA	1
GENO73	9.095	2.02	7.138	2	12	39.868	1.129	2	1
GENO74	9.688	2.421	7.283	2	26	38.095	0.884	3	1
GENO75	7.184	2.233	6.87	3	25	34.268	1.098	2	1
GENO76	8.767	2.199	7.2	2	24	38.445	0.94	1	1
GENO77	8.934	2.228	6.831	2	15	37.619	0.878	1	1
GENO78	10.478	3.727	7.013	2	32	41.719	0.6	NA	1
GENO79	9.735	3.655	6.105	2	32	32.795	0.787	1	1
GENO80	9.061	1.728	7.494	2	21	38.197	0.994	2	1
GENO81	9.31	2.542	6.753	4	13	33.471	0.97	2	1
GENO82	7.933	2.498	7.034	3	32	43.847	0.933	1	2

APPENDIX-A (Continue) Phenotypic data of nine (9) traits for the year 2016

Genotypes	Lpist	LSAA	Lsta	Maturity	NoLflts	PH	ST	ATS	BP
GENO83	8.974	2.451	6.586	4	26	60.197	1.064	1	2
GENO84	9.861	2.51	7.323	2	25	42.519	0.811	1	2
GENO85	8.79	1.85	6.925	4	32	44.671	0.584	1	2
GENO86	8.262	2.281	5.995	3	45	45.999	0.936	NA	1
GENO87	8.248	2.097	6.183	2	27	42.595	1.134	2	1
GENO88	10.244	2.744	7.625	2	24	47.419	0.902	1	2
GENO89	7.644	3.125	6.956	2	24	40.168	0.946	1	2
GENO90	8.254	2.11	6.361	2	32	48.119	0.576	1	1
GENO91	9.558	3.521	6.017	2	11	36.98	1.135	2	1
GENO92	7.815	1.59	8.19	3	18	54.097	0.904	2	2
GENO93	9	1.653	7.361	2	33	41.299	1.091	1	1
GENO94	9.506	2.496	6.995	2	26	34.071	0.764	1	2
GENO95	8.921	1.821	6.953	2	26	44.08	1.216	3	1
GENO96	9.67	2.238	7.273	2	26	33.588	0.834	NA	2
GENO97	7.469	1.106	6.516	2	36	27.597	0.799	2	1
GENO98	7.045	1.058	7.908	4	15	44.097	1.033	2	2
GENO99	8.698	1.589	7.223	3	18	36.095	0.85	1	1
GENO100	8.874	2.123	6.819	2	29	31.219	0.685	1	1
GENO101	7.711	1.916	8.056	4	33	50.497	1.056	1	2
GENO102	9.284	2.397	6.901	2	19	44.299	0.784	1	2
GENO103	9.916	2.371	7.526	2	26	47.997	0.968	1	2
GENO104	9.241	3.043	6.464	4	24	34.797	0.742	2	1
GENO105	9.454	2.847	6.707	2	18	40.399	0.711	1	2
GENO106	8.305	1.946	6.349	5	24	36.119	1.238	1	1
GENO107	8.408	1.388	7.005	5	17	30.571	0.853	2	1
GENO108	8.12	1.979	6.157	3	23	37.595	1.138	1	1
GENO109	9.039	2.767	5.603	2	17	37.491	1.175	1	1
GENO110	6.866	2.189	6.534	4	23	38.968	0.97	1	1
GENO111	8.82	1.552	7.253	2	31	44.571	0.892	NA	2
GENO112	8.534	1.962	6.557	3	22	29.071	0.806	1	1
GENO113	8.834	3.371	5.503	3	29	37.499	1.179	1	1
GENO114	9.258	2.429	6.805	3	36	36.68	0.948	1	1
GENO115	9.477	3.233	6.558	5	19	49.868	1.119	2	1
GENO116	8.292	1.627	6.717	4	31	38.399	1.203	1	1
GENO117	8.594	1.888	6.691	2	25	36.771	0.773	1	1
GENO118	9.272	1.008	8.249	4	17	45.371	0.784	2	2
GENO119	9.934	2.075	7.814	3	20	39.847	0.858	1	1
GENO120	9.094	3.347	5.999	3	35	37.899	0.884	1	2
GENO121	9.694	2.653	6.978	2	24	42.327	1.024	2	1
GENO122	8.063	1.958	8.086	3	16	44.297	1.027	1	1
GENO123	8.331	3.072	7.374	2	22	41.147	1.025	2	1
GENO124	8.629	1.931	6.603	3	34	30.519	0.795	2	2

APPENDIX-A (Continue) Phenotypic data of nine (9) traits for the year 2016

Genotypes	Lpist	LSAA	Lsta	Maturity	NoLflts	PH	ST	ATS	BP
GENO125	12.335	4.724	7.703	2	25	81.799	1.288	2	2
GENO126	8.476	2.855	5.495	4	16	36.188	0.855	2	1
GENO127	9.164	1.49	7.926	2	24	39.247	1.115	1	1
GENO128	6.383	1.81	6.218	3	34	32.097	0.994	2	1
GENO129	8.312	1.495	6.485	2	18	45.391	1.061	1	1
GENO130	10.039	2.646	7.383	3	18	38.319	0.832	2	1
GENO131	8.26	1.735	6.592	2	30	42.468	1.022	1	2
GENO132	8.624	2.383	6.255	2	17	34.199	0.808	1	1
GENO133	8.938	2.621	6.307	3	25	45.199	1.435	2	1
GENO134	6.857	2.636	5.892	4	26	63.597	1.026	2	2
GENO135	8.982	2.556	6.517	2	24	53.219	1.001	1	2
GENO137	6.233	0.96	6.85	5	25	38.647	1.241	1	1
GENO138	6.803	2.238	6.252	2	24	43.647	0.97	1	1
GENO139	9.894	2.694	6.987	2	17	53.488	0.973	2	2
GENO140	8.4	2.771	5.637	3	20	34.68	1.114	2	1
GENO141	7.365	2.08	7.262	4	20	28.447	1.067	1	1
GENO142	7.89	1.869	6.037	4	26	41.595	0.813	1	1
GENO143	11.14	3.885	7.192	4	23	33.327	1.182	1	1
GENO144	7.257	3.342	6.162	3	18	28.197	0.886	1	1
GENO145	7.031	2.216	6.648	3	18	33.197	0.759	1	2
GENO146	8.796	2.108	6.673	4	20	33.771	1.025	2	1
GENO147	9.928	2.563	7.381	2	19	59.895	1.366	2	1
GENO148	9.038	1.767	7.135	4	28	61.595	0.898	1	1
GENO149	10.236	3.391	6.861	2	24	36.795	1.161	NA	1
GENO150	10.603	2.527	8.297	5	22	52.095	1.046	1	1
GENO151	9.504	2.545	6.899	2	28	43.58	1.011	2	1
GENO152	10.795	2.355	8.325	3	30	35.895	1.193	2	1
GENO153	8.01	1.641	6.385	2	26	36.095	0.921	2	1
GENO154	8.095	1.583	6.459	5	23	20.991	1.105	1	1
GENO155	8.652	1.824	6.813	5	33	35.271	1.039	1	1
GENO156	7.006	2.309	6.672	3	22	39.368	1.437	2	2
GENO157	7.51	2.058	5.577	2	30	49.319	0.884	1	1
GENO158	8.41	1.525	6.899	2	34	46.099	0.859	1	2
GENO159	7.194	2.359	6.932	3	20	46.368	1.173	2	1
GENO160	9.226	3.051	6.189	2	25	45.399	1.063	2	1
GENO161	8.616	2.468	5.935	3	20	35.088	0.656	1	1
GENO162	6.95	1.51	5.425	3	25	42.971	1.024	2	1
GENO163	9.582	2.723	6.839	3	30	34.18	0.988	1	1
GENO164	6.894	3.137	5.864	4	12	31.468	1.178	2	1
GENO165	8.882	0.755	8.2	2	35	50.068	1.224	2	1
GENO166	5.957	2.118	5.45	2	27	52.047	1.289	2	2
GENO167	8.868	1.859	7.027	2	26	39.999	1.065	1	1

APPENDIX-A (Continue) Phenotypic data of nine (9) traits for the year 2016

Genotypes	Lpist	LSAA	Lsta	Maturity	NoLflts	PH	ST	ATS	BP
GENO168	9.397	1.951	7.393	2	29	47.591	1.11	2	2
GENO169	9.63	1.554	7.747	2	26	34.299	0.946	1	1
GENO170	9.645	2.869	6.723	2	20	37.391	1.167	3	1
GENO171	9.352	2.316	7.161	2	15	54.019	1.171	1	2
GENO172	9.258	1.689	7.549	2	49	48.28	1.227	2	2
GENO173	9.001	2.136	6.924	2	34	32.897	1.144	1	1
GENO174	8.679	2.225	6.52	2	40	29.268	1.026	2	1
GENO175	7.703	1.978	5.715	2	28	43.119	1.203	3	1
GENO176	9.888	2.595	7.23	2	32	52.727	1.082	2	1
GENO177	8.624	3.33	5.33	2	23	51.647	1.03	2	1
GENO178	7.978	2.447	7.648	2	22	45.968	1.177	3	2
GENO179	8.428	3.907	4.537	4	30	44.295	0.961	1	2
GENO180	8.147	1.865	6.23	4	31	45.247	0.787	1	2
GENO181	7.468	2.149	7.37	2	23	30.168	0.986	1	1
GENO182	9.804	2.321	7.641	2	25	35.08	0.963	1	1
GENO183	10.121	2.217	7.889	2	56	37.395	1.076	2	2
GENO184	10.112	2.5	7.737	2	17	47.519	0.818	1	2
GENO185	9.236	1.487	7.729	2	18	36.28	0.888	2	2
GENO186	9.424	2.722	7.423	2	19	36.819	1.005	1	1
GENO187	8.702	2.678	6.149	2	24	49.019	0.998	1	2
GENO188	10.594	4.16	6.559	2	27	44.619	0.6	1	1
GENO189	6.543	1.152	5.377	2	14	37.295	0.9	1	1
GENO190	11.26	3.707	7.565	3	43	43.899	1.036	1	1
GENO191	9.1	1.421	7.695	2	35	39.395	0.928	2	1
GENO192	9.19	2.296	6.861	2	21	57.38	1.127	2	1
GENO193	8.632	1.883	6.493	4	19	40.091	1.186	2	1
GENO194	10.343	2.995	7.679	3	20	38.795	0.827	2	1
GENO195	9.561	2.991	6.627	2	15	47.48	0.973	2	1
GENO196	9.441	2.073	7.638	3	29	42.797	1.059	1	2
GENO197	12.148	3.831	8.239	4	41	44.68	0.951	1	2
GENO198	9.614	2.319	7.327	3	40	35.48	0.984	1	2
GENO199	8.302	2.032	6.278	2	20	48.247	0.962	2	2
GENO200	8.091	1.583	6.427	2	32	44.091	0.849	1	2
GENO201	7.87	1.42	6.575	2	23	25.619	0.722	1	1
GENO202	9.675	3.611	6.255	2	16	48.199	0.776	1	1
GENO203	9.658	3.167	6.421	3	49	34.099	0.968	1	1
GENO204	8.415	1.861	6.717	2	17	27.48	0.91	2	1
GENO205	7.834	2.493	5.368	2	20	65.275	1.196	2	2
GENO206	6.306	1.117	5.174	2	27	60.627	1.052	2	2
GENO207	9.696	2.681	6.952	2	14	61.127	1.075	2	2
GENO208	8.187	2.192	5.949	1	29	58.819	1.209	1	2
GENO209	8.989	2.186	7.179	3	29	67.419	1.149	1	2

APPENDIX-A (Continue) Phenotypic data of nine (9) traits for the year 2016

Genotypes	Lpist	LSAA	Lsta	Maturity	NoLflts	PH	ST	ATS	BP
GENO210	8.929	2.039	6.837	2	24	59.591	1.032	2	2
GENO211	7.762	1.998	5.749	3	34	47.071	0.83	2	2
GENO212	7.813	2.457	5.392	4	20	69.327	1.342	2	1
GENO213	8.307	1.877	6.756	2	25	47.668	1.222	2	2
GENO214	9.138	3.072	6.00	2	27	62.727	1.079	2	1
GENO215	8.581	1.641	6.926	2	20	53.465	1.099	2	1
GENO216	7.878	1.646	6.253	2	30	51.667	0.945	2	2
GENO217	7.068	2.151	5.765	3	24	64.697	1.327	2	2
GENO218	6.811	1.538	5.263	2	25	40.819	0.859	1	2
GENO219	7.592	2.355	5.069	2	20	55.771	0.917	2	2
GENO220	7.75	1.404	6.206	2	28	61.289	1.192	2	2
GENO221	9.68	2.479	7.128	1	15	52.227	1.005	1	1
GENO222	8.219	2.611	5.572	1	26	42.727	0.889	1	1
GENO223	8.391	2.398	5.956	2	36	50.391	0.998	2	2
GENO224	8.314	2.537	5.714	2	28	70.327	0.871	2	1
GENO225	8.48	2.663	5.798	1	18	49.577	0.738	1	2
GENO226	7.965	2.503	5.409	2	27	66.991	1.101	3	1
GENO227	8.962	3.052	5.843	2	19	59.177	1.143	2	2
GENO228	8.414	2.43	5.961	1	22	40.591	0.876	2	1
GENO229	9.722	2.345	6.748	2	23	59.391	1.154	1	2
GENO230	8.438	2.077	6.418	2	21	51.927	1.132	2	1
GENO231	9.291	3.047	6.12	2	31	66.027	0.78	1	2
GENO232	7.926	1.778	5.907	1	25	73.788	0.991	1	2
GENO233	7.593	2.108	5.901	1	27	49.488	0.972	1	2
GENO234	8.62	2.601	5.681	2	33	75.588	1.104	2	2
GENO235	10.621	3.328	6.249	3	18	43.988	0.996	2	2
GENO236	6.841	1.438	5.177	1	20	50.088	1.098	2	2
GENO237	8.191	2.188	5.771	1	29	71.188	0.889	1	2
GENO238	7.593	2.078	5.265	2	27	58.188	1.263	2	2
GENO239	7.263	2.074	5.244	2	33	52.623	1.013	2	1
GENO240	8.691	3.831	4.88	1	17	56.75	1.017	1	1

Appendix-B Phenotypic data of eleven (11) traits for the year 2016

Genotype	Flr clr	GH	Leaf Shape	TFC	TS	TSC	TST	NTP	Yield	DMC	SG
GENO42	3	2	2	3	3	3	2	2	200.7	18.524	1.073
GENO43	3	1	1	4	2	3	1	5	522.7	21.705	1.078
GENO44	4	1	1	5	2	3	2	4	481.6	19.62	1.078
GENO45	3	2	2	5	3	3	2	5	353.5	19.685	1.079
GENO46	3	1	1	NA	NA	NA	NA	3	351.1	22.845	1.084
GENO47	1	2	1	1	2	1	1	4	343.4	19.752	1.083
GENO48	1	2	1	5	2	1	2	4	458.4	19.025	1.076
GENO49	1	2	1	2	3	1	1	5	426.5	16.582	1.063
GENO50	NA	2	1	1	3	1	1	9	862.7	23.555	1.085
GENO51	2	2	1	1	1	10	1	4	337.6	NA	NA
GENO52	1	2	1	1	1	1	2	4	358.9	21.072	1.085
GENO53	1	1	1	2	1	1	1	3	191.6	19.87	1.08
GENO54	1	1	1	7	3	1	1	2	196.4	19.08	1.073
GENO55	1	1	1	7	3	1	1	5	380.8	23.09	1.092
GENO56	3	1	2	1	2	3	1	3	246.3	20.04	1.08
GENO57	3	2	1	1	2	7	1	1	192.4	19.875	1.08
GENO58	1	2	1	1	3	1	1	4	175	19.166	1.076
GENO59	7	1	1	7	3	1	1	4	183.9	18.356	1.077
GENO60	7	2	2	7	3	1	1	4	270.2	24.935	1.094
GENO61	1	1	1	7	3	1	1	3	386.6	20.9	1.084
GENO62	9	2	1	7	3	7	2	6	355.4	23.435	1.097
GENO63	1	2	1	5	3	1	2	4	240.1	20.853	1.083
GENO64	1	1	2	1	3	1	2	3	266.8	20.085	1.081
GENO65	1	1	1	4	3	1	1	3	291.7	19.913	1.081
GENO66	1	1	1	7	2	1	1	5	344.5	22.802	1.093
GENO67	1	1	1	1	3	1	1	2	232.2	22.853	1.095
GENO68	1	1	1	1	2	1	2	2	284.9	21.335	1.087
GENO70	1	1	2	7	3	1	1	3	297.1	22.555	1.092
GENO71	1	1	1	7	2	1	1	4	375.1	NA	NA
GENO72	4	1	1	7	3	1	2	3	348.9	19.73	1.076
GENO73	1	1	1	4	3	1	1	4	459.8	21.065	1.075
GENO74	1	2	1	7	2	1	2	4	367.5	20.616	1.083
GENO75	6	1	1	4	1	1	2	4	364.2	23.365	1.086
GENO76	2	2	1	3	3	1	2	3	45.96	19.806	1.079
GENO77	1	1	1	7	1	1	1	1	175	NA	NA
GENO78	1	1	1	NA	NA	NA	NA	2	177	NA	NA
GENO79	1	1	1	7	3	NA	1	2	24.05	20.366	1.082
GENO80	1	1	1	7	2	1	1	4	368.3	20.3	1.082
GENO81	1	2	1	7	2	1	1	6	435.4	22.405	1.092
GENO82	1	2	1	1	3	1	2	3	1421.9	22.742	1.093
GENO83	1	2	1	1	3	1	1	5	278.3	23.23	1.095

APPENDIX-B (Continue) Phenotypic data of eleven (11) traits for the year 2016

Genotype	Flr clr	GH	Leaf Shape	TFC	TS	TSC	TST	NTP	Yield	DMC	SG
GENO84	4	2	1	1	3	1	2	2	123.9	20.21	1.078
GENO85	3	2	1	7	2	8	2	4	260.4	22.655	1.093
GENO86	2	1	1	NA	NA	NA	NA	2	182.2	NA	NA
GENO87	4	1	1	1	2	1	1	7	357.5	19.606	1.078
GENO88	1	1	1	2	2	1	1	3	215.3	NA	NA
GENO89	1	2	1	1	1	1	1	3	252.7	25.835	1.098
GENO90	1	1	1	7	3	1	1	2	213.7	NA	NA
GENO91	1	1	1	1	3	1	1	8	305.3	20.38	1.082
GENO92	1	2	1	7	3	1	2	3	161.5	21.73	1.088
GENO93	1	1	2	6	1	1	1	2	221.1	NA	NA
GENO94	1	2	1	7	3	1	1	2	120.4	22.715	1.094
GENO95	1	1	1	5	3	1	1	3	394.9	21.75	1.088
GENO96	1	2	1	5	2	1	2	4	107.6	23.957	1.099
GENO97	8	1	1	1	2	1	1	2	31.62	18.82	1.075
GENO98	3	1	1	7	3	3	2	3	178.3	27.65	1.116
GENO99	1	1	1	7	3	1	2	3	70.67	22.416	1.092
GENO100	1	1	1	2	3	1	2	2	227.4	21.965	1.09
GENO101	1	2	1	1	2	1	2	2	61.62	22.74	1.093
GENO102	4	2	1	1	3	1	NA	2	243.6	18.233	1.073
GENO103	1	1	1	7	3	1	1	3	55.29	22.91	1.094
GENO104	4	1	1	1	3	6	2	2	39.96	21.96	1.089
GENO105	1	1	1	2	2	1	1	3	227.4	21.693	1.09
GENO106	1	1	2	2	3	1	2	4	123.9	27.38	1.112
GENO107	1	1	1	2	2	1	1	5	326.4	26.215	1.11
GENO108	1	1	2	7	3	1	1	11	447.6	21.106	1.086
GENO109	1	1	1	3	2	1	1	3	176.6	20.324	1.091
GENO110	3	2	1	7	3	8	2	5	367.7	23.815	1.088
GENO111	2	2	1	1	2	3	1	5	423.1	22.315	1.092
GENO112	9	1	1	7	2	1	2	3	115.4	24.505	1.102
GENO113	9	1	2	4	3	3	2	2	215.8	20.193	1.083
GENO114	4	1	1	1	2	1	2	5	294.1	21.73	1.088
GENO115	1	2	1	7	3	1	1	8	712.2	26.285	1.1
GENO116	1	1	1	NA	2	10	1	1	206.1	21.393	1.088
GENO117	1	1	2	1	3	1	1	2	130.4	21.575	1.088
GENO118	1	2	1	7	2	1	2	1	166.6	20.675	1.084
GENO119	4	2	1	1	2	NA	1	4	204	22.042	1.089
GENO120	3	1	1	3	3	6	2	4	297	19.323	1.086
GENO121	4	1	1	1	3	1	1	3	129.9	21.343	1.086
GENO122	4	1	1	7	3	1	1	2	0	21.16	1.086
GENO123	1	1	1	7	3	1	2	3	395	21.262	1.086
GENO124	1	2	1	1	3	1	1	1	91.92	22.52	1.089

APPENDIX-B (Continue) Phenotypic data of eleven (11) traits for the year 2016

Genotype	Flr clr	GH	Leaf Shape	TFC	TS	TSC	TST	NTP	Yield	DMC	SG
GENO125	1	1	1	3	3	1	2	2	257.4	22.363	1.093
GENO126	1	2	1	7	3	1	2	0	193.2	22.597	1.092
GENO127	1	1	1	1	3	1	1	2	170.1	20.312	1.081
GENO128	10	1	1	7	3	10	1	3	185	24.06	1.099
GENO129	2	1	1	6	3	3	2	3	91.55	20.784	1.083
GENO130	2	1	1	5	3	1	2	3	203.9	21.02	1.082
GENO131	1	2	1	1	1	1	2	4	262.7	22.065	1.08
GENO132	1	1	1	1	3	10	1	1	180.1	NA	NA
GENO133	1	2	1	1	2	1	1	4	357	21.223	1.088
GENO134	9	2	1	1	3	3	2	4	288	20.99	1.085
GENO135	5	2	1	1	3	3	1	4	294.5	21.625	1.088
GENO137	1	1	1	1	2	1	2	4	416.9	27.462	1.115
GENO138	3	2	1	7	3	1	1	3	286.2	19.702	1.078
GENO139	4	2	1	1	3	1	2	7	185.2	23.427	1.096
GENO140	1	1	1	1	3	1	1	5	311.3	22.57	1.092
GENO141	1	1	1	7	3	1	1	5	235.8	20.122	1.08
GENO142	1	1	1	1	3	1	1	3	135.1	20.406	1.082
GENO143	1	1	1	1	3	1	2	7	162	17.903	1.069
GENO144	1	1	1	1	3	1	2	4	57.26	20.82	1.084
GENO145	1	1	1	7	3	1	1	1	0	24	1.099
GENO146	1	2	1	7	3	1	1	5	535.4	25.075	1.105
GENO147	2	2	1	1	3	1	2	6	347.2	21.066	1.085
GENO148	1	1	1	7	3	1	1	4	66.22	23.616	1.097
GENO149	1	1	1	NA	NA	NA	NA	6	87.34	19.336	1.077
GENO150	4	1	1	1	3	5	2	7	184.8	21.316	1.087
GENO151	1	1	1	1	3	5	2	6	212.8	20.1	1.081
GENO152	2	1	1	1	3	3	2	3	242.5	23.386	1.096
GENO153	1	1	1	5	3	1	1	2	174.4	18.656	1.074
GENO154	3	1	1	3	3	1	2	6	214.5	19.304	1.076
GENO155	1	2	1	5	2	1	1	5	530.4	25.365	1.106
GENO156	3	2	1	1	3	NA	1	3	447.7	22.945	1.084
GENO157	1	1	2	1	3	1	1	5	296.2	17.455	1.066
GENO158	1	2	2	3	2	1	1	2	244.9	20.083	1.082
GENO159	1	1	1	7	3	1	2	5	547.7	21.555	1.078
GENO160	2	1	1	6	2	1	1	3	326.6	21.803	1.091
GENO161	3	1	1	1	3	1	1	6	65.17	22.537	1.092
GENO162	1	2	1	1	3	1	1	4	290.4	21.085	1.091
GENO163	2	1	1	5	3	3	1	5	439.6	18.9	1.075
GENO164	1	1	1	7	3	1	2	3	356.6	20.875	1.074
GENO165	1	1	1	2	1	1	1	7	602.7	20.815	1.074
GENO166	4	2	2	1	1	1	1	5	739.5	22.022	1.089

APPENDIX-B (Continue) Phenotypic data of eleven (11) traits for the year 2016

Genotype	Flr clr	GH	Leaf Shape	TFC	TS	TSC	TST	NTP	Yield	DMC	SG
GENO167	9	1	1	1	2	1	1	6	659.3	20.153	1.083
GENO168	1	2	1	3	3	1	1	2	115.8	18.814	1.074
GENO169	1	1	1	6	3	1	1	3	326.6	20.193	1.083
GENO170	1	1	1	2	2	1	1	2	292.7	19.574	1.078
GENO171	3	2	2	4	3	1	2	1	216.3	22.235	1.091
GENO172	1	2	1	1	3	1	2	2	349.1	19.32	1.077
GENO173	4	1	1	7	1	3	1	3	86.39	22.51	1.092
GENO174	4	1	1	5	1	3	1	3	399.3	20.605	1.073
GENO175	1	2	1	2	3	1	2	4	408.9	24.53	1.099
GENO176	1	2	1	1	1	1	1	3	263	20.963	1.084
GENO177	3	2	1	1	2	1	1	5	504.3	19.552	1.078
GENO178	1	2	1	NA	1	1	1	6	487.7	22.885	1.084
GENO179	1	2	1	1	3	1	2	4	245	22.306	1.091
GENO180	1	1	1	1	NA	1	2	5	278.4	20.332	1.087
GENO181	3	1	1	5	3	11	NA	2	162.5	NA	NA
GENO182	1	1	1	1	3	1	1	3	286.6	19.79	1.079
GENO183	1	2	1	1	2	3	1	3	138	20.096	1.081
GENO184	1	2	1	1	3	1	1	3	338.7	NA	NA
GENO185	1	2	1	7	2	3	1	4	208.3	23.14	1.095
GENO186	1	1	1	1	3	1	2	1	180.1	NA	NA
GENO187	1	2	1	7	3	1	1	2	209.9	NA	NA
GENO188	1	1	1	5	3	1	1	1	201.7	NA	NA
GENO189	1	1	1	7	3	1	2	2	22.47	20.576	1.083
GENO190	1	1	1	5	2	1	2	2	199.9	NA	NA
GENO191	1	1	1	7	3	1	1	3	197.5	21.016	1.085
GENO192	1	2	1	1	3	1	1	4	375.5	21.33	1.086
GENO193	1	1	1	6	3	1	2	3	179.1	23.994	1.099
GENO194	1	1	1	7	3	1	2	3	165.9	23.746	1.098
GENO195	1	1	1	1	1	1	2	2	159.6	21.28	1.086
GENO196	1	2	1	1	2	1	1	2	53.17	20.61	1.083
GENO197	4	2	1	1	1	3	2	3	121.6	26.49	1.111
GENO198	7	1	1	NA	3	10	NA	2	101.7	23.44	1.096
GENO199	1	1	1	1	3	5	1	4	286.9	21.262	1.086
GENO200	6	2	1	1	3	10	2	2	22.98	21.324	1.086
GENO201	1	1	1	2	3	3	2	3	279.2	NA	NA
GENO202	9	2	1	1	3	5	1	3	277.8	22.433	1.093
GENO203	6	1	1	6	3	5	1	2	210.4	22.833	1.095
GENO204	1	1	1	7	2	1	1	3	170.6	17.21	1.067
GENO205	1	2	1	3	2	2	1	5	353.5	18.528	1.072
GENO206	2	2	1	7	3	3	1	6	563.8	17.333	1.067
GENO207	1	2	1	7	2	1	1	6	633.1	17.253	1.066

APPENDIX-B (Continue) Phenotypic data of eleven (11) traits for the year 2016

Genotype	Flr clr	GH	Leaf Shape	TFC	TS	TSC	TST	NTP	Yield	DMC	SG
GENO208	1	2	1	2	3	1	2	4	168.9	17.53	1.066
GENO209	3	2	1	7	2	3	1	6	503.9	21.78	1.086
GENO210	1	2	1	1	3	1	1	7	421.8	22.004	1.089
GENO211	1	2	1	1	3	1	2	6	510.4	22.785	1.094
GENO212	1	2	1	2	3	1	2	4	155.2	19.403	1.076
GENO213	1	2	1	1	2	1	2	4	492.7	23.765	1.088
GENO214	2	2	1	1	2	3	1	6	498.8	20.923	1.084
GENO215	3	2	1	4	2	3	1	6	544	20.343	1.081
GENO216	3	2	1	2	3	1	2	5	361.2	18.558	1.073
GENO217	1	2	1	4	3	1	2	5	271	25.855	1.108
GENO218	3	2	1	4	3	1	2	6	303.9	24.91	1.101
GENO219	4	2	1	2	3	1	1	7	561.4	24.355	1.101
GENO220	1	2	1	1	3	1	1	3	323.7	19.085	1.075
GENO221	1	2	1	1	3	1	2	5	331.3	19.933	1.079
GENO222	1	2	1	2	3	3	1	2	35.81	20.183	1.08
GENO223	1	2	1	2	3	1	1	7	433.2	22.089	1.089
GENO224	1	2	1	2	3	1	2	8	479.3	22.043	1.089
GENO225	1	2	1	2	3	3	1	4	160.3	21.983	1.089
GENO226	4	2	1	1	3	1	2	7	465.6	23.084	1.094
GENO227	1	2	1	4	3	6	2	5	432.1	19.778	1.078
GENO228	1	1	2	1	2	1	1	3	171.9	22.959	1.093
GENO229	1	2	1	1	3	1	1	5	406.6	18.504	1.073
GENO230	1	2	1	1	3	1	2	5	474.6	22.613	1.092
GENO231	1	2	1	4	3	1	2	7	316.3	23.843	1.097
GENO232	1	2	1	1	3	1	2	5	97.17	16.797	1.065
GENO233	1	2	1	1	3	1	2	6	135.7	19.137	1.076
GENO234	4	2	1	2	3	1	1	5	210.4	20.427	1.082
GENO235	3	2	1	1	3	1	1	6	279.4	22.627	1.092
GENO236	3	2	1	1	2	1	2	3	245.7	18.737	1.074
GENO237	3	2	1	1	3	1	2	5	113.2	17.067	1.066
GENO238	3	2	1	5	3	1	2	4	180.7	19.197	1.076
GENO239	1	2	1	3	3	1	2	5	414.8	19.844	1.077
GENO240	1	2	1	3	2	1	2	5	350	17.212	1.074

APPENDIX-C Phenotypic data of eleven (11) traits for the year 2017

Genotypes	Flr clr	LS	GH	BP	PH	NL	ST	LOS	AL	PLAS	TSC
GENO1	4	1	2	2	58.36	29	9.2	9.44	5.94	3.47	1
GENO2	1	1	2	1	44.11	28	11.89	8.32	5.49	2.79	1
GENO3	1	1	1	2	50.26	21	9.72	10.94	6.67	4.69	1
GENO4	1	1	2	1	41.36	25	12.11	8.56	4.75	3.78	1
GENO5	1	1	1	1	41.17	19	9.97	7.65	4.63	2.89	NA
GENO6	1	1	2	2	39.51	23	10.31	10.51	6.44	4.5	1
GENO7	1	1	2	2	48.36	18	12.55	8.68	5.68	2.96	1
GENO8	1	2	2	1	48.86	37	10.69	7.41	4.67	1.97	1
GENO9	1	1	2	1	49.02	17	12.24	8.89	6.17	2.83	1
GENO10	4	2	1	2	59.51	35	11.87	8.17	6.92	1.68	1
GENO11	4	1	1	1	45.02	23	11.42	8.21	6.39	1.92	3
GENO12	1	1	1	1	49.17	26	11.62	8.76	6.7	1.93	1
GENO13	1	1	2	1	56.41	36	10.92	7.62	5.32	2.44	1
GENO14	4	1	1	1	47.07	20	9.8	6.81	5.72	0.96	1
GENO15	3	1	2	2	36.36	28	8.63	10.12	4.81	5.28	6
GENO16	2	1	1	1	49.02	20	9.54	8.19	5.81	2.48	1
GENO17	3	1	1	1	58.51	24	11.11	8.74	6.02	3.14	1
GENO18	3	1	2	1	54.67	32	10.3	9.28	6.16	2.99	6
GENO19	4	1	1	1	43.61	26	8.64	9.41	5.55	3.83	6
GENO20	2	1	2	2	56.87	23	10.39	9.66	6.17	3.35	3
GENO21	2	1	2	2	60.51	23	9.42	8.19	7.66	0.95	10
GENO22	3	1	2	1	50.76	31	10.65	9.76	6.78	3.4	6
GENO23	3	1	2	2	64.02	29	10.63	8	5.23	2.88	6
GENO24	1	1	2	1	41.51	26	9.82	9.36	6.28	3.5	1
GENO25	1	1	1	1	50.52	22	9.88	8.61	6.76	1.95	1
GENO26	2	1	1	1	58.02	36	13.33	9.98	6.49	3.6	NA
GENO27	1	1	1	1	57.01	41	10.19	8.61	7.38	1.65	1
GENO28	1	1	1	2	59.51	19	10.38	9.74	6.29	3.42	1
GENO29	10	1	1	1	68.91	25	10.84	8.97	6.09	3.02	1
GENO30	2	1	1	1	42.61	23	10.2	8.07	5.36	1.93	1
GENO31	2	1	1	1	61.15	28	NA	7.79	6.96	1.1	1
GENO32	1	1	1	1	74.51	21	9.66	7.76	4.38	2.6	1
GENO33	2	1	2	1	56.01	24	11.55	7.84	6.17	2.09	1
GENO34	1	1	2	2	61.17	35	9.12	10.63	6.95	3.54	1
GENO35	2	1	2	2	72.26	21	9.58	9.34	5.7	4.06	3
GENO36	3	1	1	2	42.67	23	8.94	8.48	4.92	3.43	2
GENO37	2	1	1	1	NA	NA	NA	NA	NA	NA	1
GENO38	1	1	2	1	56.52	22	12.5	8.62	6.78	1.95	1
GENO39	2	1	2	2	68.11	26	11.81	7.88	6.01	1.83	1
GENO40	2	1	1	1	42.56	26	11.24	10.17	6.86	2.53	1
GENO41	2	1	1	1	60.86	23	9.91	9.85	5.11	3.96	3
GENO42	3	2	2	2	52.51	25	9.92	9.96	8.53	1.85	3
GENO43	3	1	1	1	65.65	25	NA	8.96	7.81	1.42	3
GENO44	4	1	1	1	79.91	34	12.56	9.92	6.34	3.72	3

APPENDIX-C (Continue) Phenotypic data of eleven (11) traits for the year 2017

Genotypes	Flr clr	LS	GH	BP	PH	NL	ST	LOS	AL	PLAS	TSC
GENO45	3	2	2	1	57.51	35	12.46	9.22	7.04	2.6	3
GENO46	3	1	1	1	37.61	19	9.82	7.22	4.95	2.23	1
GENO47	1	1	2	2	61.61	24	10.6	8.84	5.86	2.21	1
GENO48	1	1	2	1	69.91	37	12.6	9.44	6.78	2.79	1
GENO49	1	1	2	2	48.11	25	10.84	10.53	7.52	3.14	1
GENO50	1	1	2	1	62.05	20	NA	9.01	5.85	3.43	1
GENO51	5	1	2	2	55.65	27	NA	9.93	6.5	3.7	3
GENO52	1	1	2	2	65.91	28	10.13	9.2	7.18	2.16	1
GENO53	1	1	1	1	51.36	23	10.52	9.12	6.37	2.71	1
GENO54	1	1	1	1	51.76	22	11.39	8.75	5.69	2.29	1
GENO55	1	1	1	1	56.11	26	11.34	8.79	5.13	3.63	1
GENO56	3	2	2	2	42.86	26	10.75	8.98	5.04	3.17	3
GENO57	3	1	1	2	59.51	39	10.74	6.87	5.52	1.78	6
GENO58	1	1	2	1	43.65	24	NA	8.7	5.91	3.06	1
GENO59	7	1	1	1	71.41	26	10.98	7.96	5.45	2.65	1
GENO60	3	1	1	1	58.86	19	8.21	8.4	5.31	3.06	1
GENO61	1	1	1	1	55.86	28	10.43	7.45	5.07	1.6	1
GENO62	9	1	2	1	52.17	19	10.77	10.02	6.54	3.35	7
GENO63	1	1	2	1	60.52	23	10.17	7.9	5.43	2.58	1
GENO64	1	2	1	2	53.01	44	13.23	8.74	6.42	2.74	1
GENO65	1	1	1	1	47.17	38	11.92	10.19	6.05	4.02	1
GENO66	1	1	1	1	56.97	21	12	7.12	5.75	1.5	1
GENO67	1	1	1	1	46.71	38	10.81	10.08	5.65	3.65	1
GENO68	1	1	1	2	73.86	33	10.07	9.44	6.27	3.14	1
GENO69	1	1	1	1	69.92	27	13.01	10.46	6.86	3.48	1
GENO70	1	2	1	1	67.11	38	14.02	8.48	6.21	2.23	1
GENO71	1	1	1	1	48.01	25	11.68	7.76	5.37	2.81	1
GENO72	4	1	1	1	56.41	17	10.99	10.71	7.17	3.68	1
GENO73	1	1	1	1	59.81	18	14.05	7.53	4.77	1.98	1
GENO74	1	1	2	1	64.21	27	8.96	8.03	5.85	2.32	1
GENO75	9	1	2	2	65.14	26	10.8	9.85	6.65	3.46	3
GENO76	1	1	1	1	47.02	20	11.07	9.2	6.35	2.96	1
GENO77	1	1	1	1	45.17	26	12.05	9.88	6.42	3.33	1
GENO78	1	1	1	2	48.67	36	7.8	8.97	6.79	2.06	1
GENO79	1	1	1	1	NA	NA	NA	NA	NA	NA	1
GENO80	1	1	1	1	50.86	22	10.04	9.44	5.2	3.46	1
GENO81	1	1	2	1	48.17	18	11.05	9.38	6	3.25	1
GENO82	1	1	1	1	50.17	21	10.55	10.11	9.98	3.74	1
GENO83	1	1	2	2	60.36	29	9.91	9.11	5.59	3.49	1
GENO84	4	1	2	2	48.17	26	9.25	9.8	6.9	2.77	1
GENO85	3	1	2	2	55.17	32	8.19	7.78	6.44	1.21	8
GENO87	4	1	1	1	53.46	31	12.58	9.65	6.2	2.67	1
GENO88	1	1	1	1	40.17	28	12.19	11.07	7.97	2.96	1
GENO89	1	1	2	2	53.67	26	9.41	10.41	5.87	4.41	1
GENO90	1	1	1	1	53.67	35	8.56	9.86	6.84	2.89	1
GENO91	1	1	1	1	50.36	19	12.15	8.86	4.87	3.95	1

APPENDIX-C (Continue) Phenotypic data of eleven (11) traits for the year 2017

Genotypes	Flr clr	LS	GH	BP	PH	NL	ST	LOS	AL	PLAS	TSC
GENO92	1	1	2	2	70.91	20	9.45	9.13	7.18	2.09	1
GENO93	1	2	1	1	49.52	34	12.29	9.23	6.97	2.36	1
GENO94	1	1	2	2	49.59	23	9.99	8.5	6.42	2.22	1
GENO95	1	1	1	1	58.41	31	12.95	7.32	5.72	1.74	1
GENO96	1	1	2	2	62.91	25	9.99	8.67	6.63	2.18	1
GENO97	7	2	2	2	61.67	26	11.3	10.55	7.22	3.21	1
GENO98	3	1	1	2	59.67	20	10.57	9.76	7.82	1.81	3
GENO99	1	1	1	1	56.9	24	NA	8.49	6.23	2.53	1
GENO100	1	1	2	1	67.01	28	14.25	9.61	6.29	3.75	1
GENO101	1	1	2	2	50.02	34	10.35	8.11	6.1	2.12	1
GENO102	4	1	2	2	60.02	25	9.42	9.15	6.47	2.78	1
GENO103	1	1	1	2	49.75	28	NA	9.96	6.93	3.31	1
GENO104	2	1	2	2	56.9	33	NA	8.41	5.52	3.16	10
GENO105	1	1	1	2	56.02	25	10.01	10.66	7.18	3.59	1
GENO106	1	2	1	1	49.67	26	11.21	9.18	5.76	3.3	1
GENO107	1	1	1	1	51.01	19	10.88	8.28	6.7	2	1
GENO108	1	2	1	1	53.11	24	11.51	7.19	5.2	1.96	1
GENO109	1	1	1	1	47.01	23	12.12	9.96	5.4	4.98	1
GENO110	3	1	2	1	64.65	22	NA	9.46	6.3	3.43	8
GENO111	2	1	2	2	68.17	32	9.27	10.72	7.7	2.89	3
GENO112	3	1	2	1	53.42	20	11.76	7.41	5.34	1.95	1
GENO113	3	1	1	1	57.11	27	10.65	8.18	4.52	2.88	3
GENO114	4	1	1	1	55.02	33	10.28	8.65	6.47	2.28	1
GENO115	1	1	2	1	45.17	22	11.21	11.34	7.77	3.44	1
GENO116	1	1	1	1	43.67	34	11.93	8.71	6.82	1.76	10
GENO117	1	2	1	1	65.17	24	9.08	7.95	5.03	2.79	1
GENO118	1	1	2	2	48.52	20	9.19	9.17	6.58	2.46	1
GENO119	4	1	2	1	83.41	18	8.79	9.77	7	2.91	11
GENO120	1	1	1	1	54.36	36	9.99	8.89	6.5	2.35	1
GENO121	4	1	1	1	65.5	27	NA	9.62	6.58	3.31	1
GENO122	4	1	1	1	54.51	20	10.95	8.98	6.97	2.44	1
GENO123	1	1	1	1	54.02	21	11.71	10.2	6.77	3.54	1
GENO124	1	1	2	2	62.17	31	8.8	9.01	6.72	2.16	1
GENO125	1	1	1	2	64.91	28	12.7	13.04	7.09	6.09	1
GENO126	1	1	2	1	47.01	19	10.25	9.35	7.26	2.51	1
GENO127	1	1	1	1	55.7	24	NA	8.06	5.99	2.34	1
GENO128	10	1	1	1	67.41	36	10.56	6.5	4.53	2.11	10
GENO129	2	1	1	1	62.91	24	10.98	9.56	6.8	2.9	3
GENO130	1	1	1	1	51.67	20	9.56	10.6	7.38	3.08	1
GENO131	1	1	2	2	58.67	33	10.96	10.9	6.3	4.48	1
GENO132	1	1	1	1	50.17	22	9.45	8.52	6.05	2.34	10
GENO133	8	1	1	1	57.52	37	9.05	7.54	5.81	1.84	1

APPENDIX-C (Continue) Phenotypic data of eleven (11) traits for the year 2017

Genotypes	Flr clr	LS	GH	BP	PH	NL	ST	LOS	AL	PLAS	TSC
GENO134	10	1	2	2	62.17	36	8.86	9.2	6.95	2.12	10
GENO135	3	1	2	1	71.17	39	9.93	7.29	5.21	1.95	6
GENO137	1	2	2	1	66.67	41	10.13	8.51	6.69	1.69	3
GENO138	3	1	1	1	56.36	26	11.16	8.52	4.73	3.02	1
GENO139	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GENO140	1	1	1	1	64.36	26	11.93	9.11	5.34	3	1
GENO141	1	1	1	1	49.02	18	12.31	9.9	6.96	3.04	1
GENO142	1	1	1	1	52.3	28	NA	8.47	6.4	2.34	1
GENO143	1	1	1	1	41.01	26	13.38	10.17	7.18	3.41	1
GENO144	1	1	1	1	70.65	20	NA	8.72	5.25	3.74	1
GENO145	1	1	1	2	47.86	21	9.26	7.54	5.31	2.19	1
GENO146	2	1	2	1	43.91	20	13.78	9.47	6.15	3.46	1
GENO147	2	1	2	1	64.02	20	11.78	9.79	6.26	3.64	1
GENO148	1	1	1	1	50.1	31	10.22	8.75	6.31	2.43	1
GENO149	1	1	1	1	45.51	25	11.73	9.1	6.5	3.03	1
GENO150	4	1	1	1	59.11	23	10.59	8.78	6.83	1.92	7
GENO151	3	1	1	1	68.52	32	10.9	9.12	6.19	3.04	7
GENO152	2	1	1	1	52.86	34	13.17	8.46	4.65	3.03	3
GENO153	1	1	1	1	53.36	32	11.07	7.8	5.06	1.96	1
GENO154	3	1	1	1	37.01	26	11.42	10.4	7.09	3.73	1
GENO155	1	1	2	1	58.17	35	10.01	10.14	7.02	3	1
GENO156	3	1	2	2	52.17	26	11.61	10.01	6.12	3.76	1
GENO157	1	2	1	1	56.17	35	10.64	10.57	7.49	2.95	1
GENO158	1	2	2	2	47.02	32	10.96	7.87	6.63	1.35	1
GENO159	1	1	1	1	61.36	22	11.52	9.98	5.3	3.9	1
GENO160	2	1	1	1	51.01	29	12	6.16	4.52	2.06	1
GENO161	9	1	1	1	27.86	24	8.75	9.12	4.63	3.72	1
GENO162	1	1	2	1	51.76	24	10.93	9.86	7.26	3.02	1
GENO163	2	1	1	1	67.65	35	NA	7.84	6.16	1.95	3
GENO164	1	1	1	1	79.75	19	NA	8.49	6.87	1.9	1
GENO165	1	1	1	1	53.02	34	12.99	10.15	7.39	2.87	1
GENO166	4	2	2	2	48.52	24	10.85	9.5	6.76	2.84	1
GENO167	9	1	1	1	56.01	30	10.55	9.84	7.49	2.77	1
GENO168	1	1	2	2	35.01	32	11.47	8.6	6.99	2.03	1
GENO169	1	1	1	1	75.35	30	NA	9.56	6.98	2.85	1
GENO170	1	1	1	1	51.36	25	12.04	8.31	5.17	3.11	1
GENO171	3	2	2	2	56.71	23	NA	7.4	4.4	2.22	1
GENO172	1	1	2	2	47.86	53	12	8.15	5.58	1.79	1
GENO173	4	1	1	1	52.52	34	11.26	8.55	6.5	2.16	3
GENO174	4	1	1	1	60.36	38	11.01	8.24	5.81	2.4	3
GENO175	1	1	2	1	51.65	27	NA	8.57	5.72	3.13	1
GENO176	1	1	2	1	43.91	35	12.38	10.04	6.17	4	1

APPENDIX-C (Continue) Phenotypic data of eleven (11) traits for the year 2017

Genotypes	Flr clr	LS	GH	BP	PH	NL	ST	LOS	AL	PLAS	TSC
GENO177	1	1	1	1	55.11	25	9.5	9.64	5.86	3.01	1
GENO178	1	1	1	2	63.95	24	NA	7.86	5.78	2.35	1
GENO179	1	1	2	2	59.91	31	9.73	7.69	5.01	2.81	1
GENO180	1	1	1	2	54.52	28	10.53	7.47	5.19	2.39	1
GENO181	3	1	1	1	48.51	25	8.45	9.6	6.94	2.79	6
GENO182	1	1	1	1	58.86	30	10.42	9.28	6.2	3.04	1
GENO183	1	1	2	2	67.41	57	11.99	9.6	7.24	2.49	3
GENO184	1	1	2	2	49.9	24	NA	7.85	5.92	2.21	1
GENO185	1	1	2	2	38.17	26	9.73	8.8	6.03	2.65	3
GENO186	1	1	1	1	47.67	27	11.32	10.09	6.29	3.67	1
GENO187	1	1	2	2	63.67	30	10	9.55	6.19	3.23	1
GENO188	1	1	1	1	58.17	30	9.72	10.28	5.99	4.16	1
GENO189	1	1	1	1	43.86	16	9.12	8.95	5.89	3.03	1
GENO190	1	1	1	1	51.52	45	12.26	9.91	6.55	3.46	1
GENO191	9	1	1	1	59.67	27	10.18	8.46	6.74	1.59	3
GENO192	1	1	2	1	57.86	27	12.07	8.57	5.12	2.67	1
GENO193	1	1	1	1	55.65	21	NA	8.12	6.03	2.36	1
GENO194	1	1	2	2	54.86	30	9.54	8.36	5.75	2.58	1
GENO195	1	1	1	1	65.86	24	8.85	8.31	4.92	2.62	1
GENO196	1	1	2	2	67.41	31	11.03	8.83	5.99	2.97	1
GENO197	4	1	2	2	72.22	46	13.25	9.96	7.23	2.6	3
GENO198	7	1	1	2	64.67	46	10.25	10.03	6.63	3.26	10
GENO200	3	1	2	2	40.67	18	11.12	8.87	5.96	2.78	7
GENO201	1	1	1	1	39.01	29	10.49	8	6.38	2.04	3
GENO202	9	1	2	1	50.17	25	9.56	8.87	5.85	2.89	7
GENO203	10	1	1	1	45.17	52	11.59	8.01	5.91	1.97	5
GENO204	1	1	1	1	60.45	23	13.78	8.24	6.18	2.34	1
GENO205	1	2	2	2	61.96	29	13.77	7.87	5.98	2.21	1
GENO206	2	1	2	2	45.41	30	11.72	8.36	5.84	2.66	3
GENO207	1	1	2	2	50.01	19	12.31	10.45	7.51	3.36	1
GENO208	1	1	2	2	50.67	24	12.53	8.63	5.32	3.18	1
GENO210	1	1	2	2	64.8	27	NA	7.03	4.93	2.38	1
GENO211	1	1	2	2	65.36	33	6.65	6.7	3.39	2.54	1
GENO212	1	1	2	1	69.36	25	13.98	6.64	3.77	2.09	1
GENO213	1	1	2	2	44.91	25	10.98	9.95	7.14	2.95	1
GENO214	2	1	2	1	50.61	30	11.34	6.87	5.39	1.45	3
GENO215	3	2	2	1	60.64	24	14.32	8.52	6.82	1.7	4
GENO216	3	1	2	1	55.09	29	12.58	8.36	6.2	2.16	1
GENO217	1	1	2	2	63.46	26	13.82	8.65	5.13	3.49	1
GENO218	3	1	2	2	NA	NA	NA	NA	NA	NA	1
GENO219	4	1	2	2	NA	NA	NA	NA	NA	NA	1
GENO220	1	1	2	2	62.92	27	11.23	8.45	5.75	2.62	1

APPENDIX-C (Continue) Phenotypic data of eleven (11) traits for the year 2017

Genotypes	Flr clr	LS	GH	BP	PH	NL	ST	LOS	AL	PLAS	TSC
GENO221	1	1	2	1	63.41	18	11.61	10.48	7.06	3.55	1
GENO222	1	1	2	1	30.15	29	NA	7.71	6.84	1.14	3
GENO223	1	1	2	2	77.91	37	10.4	8.41	6.04	2.51	1
GENO224	1	1	2	1	47.65	30	NA	8.32	5.33	3.27	1
GENO225	1	1	2	2	49.91	26	10.62	8.57	5.8	2.91	3
GENO226	4	1	2	1	67.25	30	NA	8.15	5.16	3.26	1
GENO227	1	1	2	2	56.02	25	13.03	10.18	6.52	3.77	6
GENO228	1	2	1	1	51.4	26	NA	7.64	5.25	2.66	1
GENO229	1	1	2	2	59.35	28	11.87	9.46	6.41	3.32	1
GENO230	1	1	2	1	67.66	24	11.88	9.19	4.68	3.73	1
GENO231	1	1	2	2	69.44	32	9.65	10.08	6.55	3.6	1
GENO232	1	1	2	2	53.02	27	11.4	10.78	5.6	5.29	1
GENO233	1	1	2	2	46.41	26	9.62	9.02	6.89	2.26	1
GENO234	4	1	2	2	61.31	32	11.76	9.73	4.58	4.37	1
GENO235	3	1	2	2	49.91	19	9.52	10.64	6.12	4.65	1
GENO236	3	1	2	2	43.52	20	10.55	9.89	6.97	3.02	1
GENO237	3	1	2	2	62.52	26	10.32	8.21	6.13	2.18	1
GENO238	3	1	2	2	62.15	28	NA	7.78	6.34	1.72	1
GENO239	1	2	2	1	54.89	34	12.74	9.28	6.93	2.89	1
GENO240	1	1	2	1	59.69	22	13.09	8.58	4.67	3.91	1

APPENDIX-D Phenotypic data of nine (9) traits for the year 2017

Genotypes	TFC	TST	TS	TSz	NTP	TY	Maturity	DMC	SG
GENO1	1	1	2	2	5	0.2925	1	20.31	1.08
GENO2	7	2	2	2	3	0.135	1	18.21	1.071
GENO3	7	1	1	1	2	0.1759	2	15.43	1.051
GENO4	1	1	3	1	6	0.2575	1	19.48	1.077
GENO5	NA	NA	NA	1	9	0.1173	2	NA	NA
GENO6	1	1	3	3	6	0.4567	3	19.58	1.077
GENO7	1	1	3	1	4	0.23	1	18.71	1.073
GENO8	1	1	3	3	7	0.6167	1	19.06	1.075
GENO9	1	1	3	2	3	0.1963	2	17.62	1.068
GENO10	3	1	3	1	3	0.108	1	15.79	1.059
GENO11	1	1	3	3	4	0.3828	2	21.29	1.085
GENO12	1	2	NA	NA	0	0	3	NA	NA
GENO13	1	2	3	1	2	0.075	2	19.06	1.075
GENO14	5	1	3	2	24	0.8655	2	NA	NA
GENO15	2	1	3	1	1	0.02105	2	NA	NA
GENO16	7	2	2	1	0	0.01852	2	NA	NA
GENO17	7	2	3	3	8	0.5654	1	15.68	1.059
GENO18	1	1	3	1	15	0.78	2	23.34	1.095
GENO19	1	2	3	1	1	0.00571	2	NA	NA
GENO20	1	2	3	1	3	0.07624	2	NA	NA
GENO21	1	2	3	1	0	0.028	2	NA	NA
GENO22	7	2	3	2	8	0.6324	3	23	1.093
GENO23	4	2	3	1	0	0.03667	2	17.73	1.068
GENO24	7	2	1	3	1	0.09286	3	NA	NA
GENO25	1	2	3	3	4	0.3784	2	23.57	1.096
GENO26	2	1	3	1	4	0.169	3	19.69	1.078
GENO27	5	1	2	1	2	0.05862	1	20.07	1.079
GENO28	1	1	3	1	4	0.1158	1	16.33	1.062
GENO29	4	2	1	1	3	0.2083	2	19.56	1.077
GENO30	5	1	2	1	3	0.1382	2	20.715	1.0825
GENO31	5	1	2	2	5	0.345	1	17.28	1.066
GENO32	1	1	2	3	4	0.3125	2	20.34	1.081
GENO33	7	1	1	2	9	0.3962	2	17.79	1.069
GENO34	2	1	3	1	3	0.075	1	NA	NA
GENO35	1	1	3	2	5	0.3414	2	19.52	1.077
GENO36	7	1	3	2	10	0.3592	2	NA	NA
GENO37	5	1	3	3	NA	NA	NA	NA	NA
GENO38	7	2	1	1	2	0.1138	2	NA	NA
GENO39	1	2	2	3	6	0.3775	1	16.5	1.062

APPENDIX-D (Continue) Phenotypic data of nine (9) traits for the year 2017

Genotypes	TFC	TST	TS	TSz	NTP	TY	Maturity	DMC	SG
GENO40	7	1	2	3	4	0.3763	2	14.43	1.053
GENO41	1	1	2	2	8	0.4846	2	16.27	1.061
GENO42	4	2	3	3	4	0.4063	2	15.89	1.06
GENO43	4	1	2	3	7	0.5949	1	16.36	1.062
GENO44	5	2	2	3	6	0.5636	2	17.09	1.063
GENO45	5	2	3	3	5	0.363	3	18.78	1.073
GENO46	1	1	3	1	2	0.105	1	18.13	1.07
GENO47	1	1	2	3	4	0.3154	2	16.29	1.061
GENO48	5	2	2	3	5	0.37	2	14.92	1.055
GENO49	2	1	3	3	5	0.45	2	15.32	1.057
GENO50	1	1	3	2	4	0.2263	1	14.46	1.053
GENO51	1	1	3	1	2	0.09189	2	18.42	1.072
GENO52	1	2	1	3	9	0.7286	2	19.14	1.075
GENO53	2	1	3	3	3	0.3541	1	17.24	1.066
GENO54	7	1	3	3	4	0.2872	1	17.18	1.066
GENO55	7	1	3	2	4	0.2027	1	17.45	1.067
GENO56	1	1	2	2	4	0.2289	1	16.88	1.062
GENO57	1	2	3	1	6	0.3357	2	16.76	1.064
GENO58	1	1	3	3	4	0.235	1	19.39	1.076
GENO59	7	1	3	1	4	0.2162	1	17.24	1.066
GENO60	1	1	3	3	2	0.1325	2	16.61	1.063
GENO61	7	1	3	1	1	0.01538	1	20.15	1.08
GENO62	7	2	3	1	2	0.05505	2	NA	NA
GENO63	5	2	3	1	2	0.07368	2	16.04	1.06
GENO64	1	2	3	1	0	0.004	1	NA	NA
GENO65	5	1	3	1	3	0.07273	3	NA	NA
GENO66	7	1	2	2	6	0.2792	1	21.175	1.0845
GENO67	1	1	3	2	4	0.3325	2	NA	NA
GENO68	1	2	2	2	3	0.1375	1	17.58	1.068
GENO69	7	2	3	1	2	0.08	2	NA	NA
GENO70	7	1	3	1	4	0.1737	1	18.49	1.072
GENO71	7	1	2	3	2	0.2424	2	18.7	1.073
GENO72	7	2	3	3	4	0.4152	2	18	1.07
GENO73	4	1	3	2	6	0.3718	1	16.42	1.062
GENO74	7	2	2	3	4	0.3556	2	19.69	1.078
GENO75	1	2	3	1	4	0.3162	2	18.515	1.072
GENO76	1	1	3	1	5	0.2731	2	19.23	1.075
GENO77	7	1	1	1	1	0.01103	2	NA	NA
GENO78	1	2	3	1	4	0.2271	2	NA	NA

APPENDIX-D (Continue) Phenotypic data of nine (9) traits for the year 2017

Genotypes	TFC	TST	TS	TSz	NTP	TY	Maturity	DMC	SG
GENO82	5	2	1	1	3	0.1391	3	NA	NA
GENO83	1	1	3	1	3	0.07	2	19.69	1.078
GENO84	1	2	3	NA	1	0.00433	3	NA	NA
GENO85	7	2	2	1	8	0.2286	2	NA	NA
GENO87	1	1	2	2	7	0.4	1	16.76	1.064
GENO88	2	1	2	3	12	0.9045	2	19.33	1.076
GENO89	1	1	1	1	3	0.06951	2	NA	NA
GENO90	7	1	3	1	6	0.1532	2	NA	NA
GENO91	1	1	3	1	6	0.235	1	18.4	1.071
GENO92	7	2	3	2	4	0.2486	2	20.79	1.083
GENO93	7	1	1	1	1	0.05135	2	15.38	1.057
GENO94	7	1	3	3	1	0.09729	2	16.67	1.063
GENO95	5	1	3	2	2	0.2028	2	16.84	1.064
GENO96	5	2	2	1	1	0.05455	1	16.82	1.064
GENO97	7	1	3	1	2	0.1071	2	19.29	1.076
GENO98	7	2	3	1	1	0.0664	2	NA	NA
GENO99	7	2	3	1	3	0.1237	2	20.41	1.081
GENO100	1	1	2	3	5	0.471	2	17.73	1.068
GENO101	1	2	2	1	10	0.1633	2	16.12	1.061
GENO102	1	2	3	1	2	0.1192	2	16.55	1.063
GENO103	7	1	3	2	3	0.1487	2	17.58	1.068
GENO104	1	1	1	3	4	0.32	2	17.26	1.066
GENO105	1	1	2	1	2	0.08966	2	18.42	1.072
GENO106	2	2	3	1	1	0.03747	2	NA	NA
GENO107	2	1	2	2	1	0.06667	2	NA	NA
GENO108	7	1	3	1	4	0.1189	1	16.27	1.061
GENO109	4	1	2	3	4	0.2467	2	19.33	1.076
GENO110	7	2	3	1	2	0.0925	2	21.55	1.086
GENO111	1	1	2	1	8	0.3917	2	17.12	1.065
GENO112	1	1	3	1	5	0.1167	2	NA	NA
GENO113	1	2	2	3	0	0.02778	2	18.68	1.073
GENO114	1	2	2	1	3	0.155	2	19.81	1.078
GENO115	7	1	3	3	7	0.5333	2	16.78	1.064
GENO116	1	1	2	1	8	0.161	2	NA	NA
GENO117	1	1	3	1	6	0.1236	2	NA	NA
GENO118	7	2	3	1	9	0.1468	1	NA	NA
GENO119	1	1	2	1	4	0.1806	2	NA	NA
GENO120	7	1	3	3	4	0.25	2	18.99	1.074
GENO121	1	1	3	1	1	0.00174	1	NA	NA

APPENDIX-D (Continue) Phenotypic data of nine (9) traits for the year 2017

Genotypes	TFC	TST	TS	TSz	NTP	TY	Maturity	DMC	SG
GENO125	4	2	3	2	2	0.1821	2	18.8	1.073
GENO126	7	2	3	1	0	0.03667	2	NA	NA
GENO127	1	1	3	1	1	0.00513	1	NA	NA
GENO128	7	1	3	1	1	0.04103	1	18.71	1.073
GENO129	7	2	3	2	4	0.2629	2	17.66	1.068
GENO130	5	2	3	1	2	0.15	2	NA	NA
GENO131	1	2	1	1	0	0	2	NA	NA
GENO132	1	1	3	1	15	0.2119	2	NA	NA
GENO133	1	1	2	NA	4	0.3536	2	16.55	1.063
GENO134	1	2	3	1	3	0.1667	2	NA	NA
GENO135	1	2	3	1	1	0.04615	2	NA	NA
GENO137	1	2	3	1	8	0.4732	1	19.44	1.076
GENO138	7	1	3	1	2	0.09429	2	16.46	1.062
GENO139	NA	NA	NA	NA	NA	NA	NA	NA	NA
GENO140	1	1	3	1	2	0.1243	2	18.78	1.073
GENO141	7	1	3	1	2	0.08462	2	15.6	1.058
GENO142	1	1	3	1	1	0.01026	2	NA	NA
GENO143	1	2	3	1	1	0.04286	2	NA	NA
GENO144	1	2	3	NA	8	0.2316	1	15.26	1.057
GENO145	7	1	3	1	3	0.1	1	18.95	1.074
GENO146	1	2	3	2	4	0.3344	2	19.99	1.079
GENO147	1	1	3	1	1	0.05833	2	15.15	1.056
GENO148	7	1	3	1	1	0.03224	3	19.39	1.076
GENO149	1	2	3	1	3	0.1433	2	16.55	1.063
GENO150	1	2	3	1	4	0.1289	2	19.52	1.079
GENO151	1	2	3	1	2	0.08333	1	17.22	1.066
GENO152	1	2	3	2	0	0.02368	1	NA	NA
GENO153	5	2	3	1	1	0.08205	1	NA	NA
GENO154	4	2	3	1	2	0.1108	2	17.05	1.065
GENO155	5	1	2	1	2	0.09333	2	NA	NA
GENO156	1	1	3	1	2	0.1187	2	19.61	1.077
GENO157	1	1	3	2	6	0.3556	2	18.95	1.074
GENO158	4	1	3	2	11	0.8333	2	17.94	1.069
GENO159	7	2	3	1	2	0.1194	2	14.5	1.053
GENO160	7	1	2	3	5	0.5143	2	20.85	1.083
GENO161	7	2	2	1	3	0.1282	1	20.07	1.079
GENO162	1	1	3	1	3	0.2231	2	17.35	1.066
GENO163	5	1	3	1	2	0.08649	2	16.21	1.061
GENO164	7	2	3	1	2	0.08205	2	17.68	1.068

APPENDIX-D (Continue) Phenotypic data of nine (9) traits for the year 2017

Genotypes	TFC	TST	TS	TSz	NTP	TY	Maturity	DMC	SG
GENO168	4	1	3	1	1	0.05	1	14.27	1.052
GENO169	7	1	3	1	2	0.07179	3	18.21	1.071
GENO170	1	1	2	1	1	0.06	1	NA	NA
GENO171	4	2	3	3	4	0.4128	2	18.02	1.07
GENO172	1	2	3	1	0	0.01714	1	NA	NA
GENO173	7	1	1	3	4	0.48	3	20.39	1.081
GENO174	5	1	1	1	2	0.1175	1	18.36	1.071
GENO175	2	2	3	2	4	0.275	2	19.37	1.076
GENO176	1	1	1	1	4	0.2889	1	19.06	1.075
GENO177	7	2	3	1	4	0.275	1	20.09	1.079
GENO178	7	1	1	1	1	0.03158	2	16.76	1.061
GENO179	1	2	3	1	1	0.03611	2	NA	NA
GENO180	1	2	2	2	2	0.1281	2	18.3	1.071
GENO181	5	1	3	1	1	0.04333	2	NA	NA
GENO182	1	1	3	1	1	0.0225	1	NA	NA
GENO183	1	1	2	1	1	0.0325	1	NA	NA
GENO184	1	1	3	2	4	0.2641	2	18.74	1.073
GENO185	7	1	2	1	4	0.08772	2	NA	NA
GENO186	1	2	3	1	0	0.02233	2	NA	NA
GENO187	7	1	3	1	5	0.1286	2	NA	NA
GENO188	5	1	3	2	11	0.4286	2	18.17	1.07
GENO189	7	2	3	1	2	0.1103	1	18.72	1.073
GENO190	6	2	2	1	3	0.2111	2	16.19	1.061
GENO191	5	2	3	1	3	0.11	2	NA	NA
GENO192	1	1	3	2	5	0.2211	1	19.35	1.076
GENO193	7	2	3	1	3	0.1184	2	18.21	1.071
GENO194	1	2	3	NA	0	0	1	NA	NA
GENO195	1	2	1	2	3	0.185	1	16	1.06
GENO196	1	1	2	1	2	0.0825	1	NA	NA
GENO197	1	2	3	1	7	0.1	1	NA	NA
GENO198	1	2	3	1	2	0.07882	1	NA	NA
GENO200	1	1	2	1	3	0.2671	1	17.79	1.069
GENO201	2	2	3	1	1	0.04828	2	NA	NA
GENO202	1	1	3	1	10	0.1718	2	NA	NA
GENO203	7	1	3	NA	3	0.06667	3	NA	NA
GENO204	7	1	2	2	5	0.2769	1	14.98	1.055
GENO205	1	1	2	1	3	0.1561	1	16.406	1.062
GENO206	7	1	3	3	1	0.09091	1	14.92	1.055
GENO207	7	1	2	2	3	0.3	2	16.31	1.062

APPENDIX-D (Continue) Phenotypic data of nine (9) traits for the year 2017

Genotypes	TFC	TST	TS	TSz	NTP	TY	Maturity	DMC	SG
GENO212	2	2	3	1	1	0.04359	2	22.18	1.089
GENO213	1	2	2	2	3	0.2171	2	19.35	1.076
GENO214	1	1	2	1	1	0.06316	1	16.08	1.06
GENO215	1	2	2	1	3	0.1959	1	17.06	1.0653
GENO216	1	1	3	2	4	0.3028	1	15.3586	1.057
GENO217	4	1	3	1	3	0.1237	2	20.43	1.081
GENO218	4	2	3	1	NA	NA	NA	NA	NA
GENO219	2	1	3	3	NA	NA	NA	NA	NA
GENO220	1	1	3	1	3	0.1963	2	16.02	1.06
GENO221	1	2	3	1	2	0.08286	1	17.79	1.069
GENO222	2	1	3	1	2	0.1057	1	19.5	1.077
GENO223	1	1	3	NA	5	0.4333	2	19.25	1.075
GENO224	2	2	3	3	3	0.2526	1	18.89	1.074
GENO225	2	1	3	2	4	0.2235	1	22.73	1.092
GENO226	2	2	3	1	1	0.05	1	17.01	1.065
GENO227	1	2	3	1	5	0.3346	1	17.83	1.069
GENO228	2	1	2	1	4	0.1972	1	20.89	1.083
GENO229	1	1	3	2	4	0.3385	1	17.22	1.066
GENO230	1	2	3	1	3	0.125	1	18.8	1.073
GENO231	4	2	3	3	7	0.4203	1	19.435	1.0765
GENO232	1	2	2	1	2	0.1267	1	14.9	1.055
GENO233	1	2	3	2	3	0.1941	1	15.64	1.058
GENO234	2	1	3	1	2	0.16	1	17.62	1.068
GENO235	1	1	3	1	8	0.5816	2	19.63	1.077
GENO236	1	2	2	1	3	0.1926	3	16.29	1.061
GENO237	1	2	3	3	4	0.4222	1	17.56	1.067
GENO238	5	2	3	2	2	0.08421	2	15.03	1.055
GENO239	7	1	2	3	5	0.3495	2	15.9233	1.0598
GENO240	2	2	2	1	3	0.127	1	16.2286	1.0611

APPENDIX- E Name of genotypes used in thesis

Genotype Name	Genotype ID	Genotype Name	Genotype ID
01.536	1	395017.229	121
02.363	2	395017.242	122
02.173	3	395186.6	124
03.113	4	395196.4	125
04.123	5	395432.51	126
06.62	6	395436.8	128
07.258	7	395445.16	129
08.212	8	396004.337	130
300046.22	9	396009.258	131
300048.12	10	396012.266	132
300054.29	11	397016.7	133
300055.32	12	396038.101	134
300056.33	13	396038.105	135
300063.9	14	396038.105	136
300065.4	15	396063.1	137
300066.11	16	396063.16	138
300093.14	17	396180.22	139
300099.22	18	396268.1	140
300101.11	19	396268.9	141
300135.14	20	396269.14	142
300135.3	21	396269.16	143
301023.15	22	396272.12	144
301024.95	23	396272.18	145
301026.23	24	396272.21	147
301029.18	25	396272.37	148

APPENDIX- E (Continue) Name of genotypes used in thesis

Genotype Name	Genotype ID	Genotype Name	Genotype ID
301041.26	26	396273.48	149
301044.36	27	396285.1	150
301045.74	28	396287.5	151
301055.53	29	396311.1	152
302428.20	30	397006.18	153
302476.108	31	397012.20	154
302498.70	32	397029.21	156
302499.30	33	397030.31	157
303381.106	34	397035.26	158
303381.30	35	397036.7	159
304345.102	36	397039.53	160
304347.6	37	397060.19	161
304349.8	38	397067.2	162
304350.100	39	397069.5	163
304350.118	40	397073.15	164
304350.18	41	397073.7	165
304350.78	42	397078.12	167
304350.95	43	397079.26	168
304351.109	44	397098.12	169
304351.31	45	397099.4	170
304369.22	46	397099.6	171
304371.20	47	397100.9	172
304371.67	48	397196.3	173
304383.80	50	397196.8	174
304387.39	51	397197.9	175
304387.92	52	398014.2	176
304399.15	54	398098.119	177
304399.5	55	398098.205	178

APPENDIX- E (Continue) Name of genotypes used in thesis

Genotype Name	Genotype ID	Genotype Name	Genotype ID
304405.42	56	398098.231	179
304405.47	57	398098.570	180
304406.31	58	398098.65	181
374080.5	59	398180.144	182
377744.1	60	398180.253	183
379706.27	61	398180.289	184
380496.6	62	398180.612	185
381381.13	63	398190.112	186
381381.9	64	398190.404	187
384866.5	65	398190.571	188
385499.11	66	398190.615	189
385558.2	67	398193.158	190
388611.22	68	398193.650	191
387164.4	69	398208.219	192
388972.22	70	398208.29	193
389468.3	71	398208.33	194
389746.2	72	398208.620	195
390478.9	73	398208.670	196
390637.1	74	399067.22	197
390663.8	75	399078.11	198
391004.18	76	399085.23	199
391046.14	77	399085.30	200
391065.69	78	694474.33	202
391065.81	79	720072	203
391207.2	80	800258	204
391382.18	81	Agria	205
391580.30	82	Balatoni Rozsa	206
391583.25	83	Bamba	207

APPENDIX- E (Continue) Name of genotypes used in thesis

Genotype Name	Genotype ID	Genotype Name	Genotype ID
391585.179	84	Chipke	208
391585.5	85	CIP24	209
391691.96	86	CIP 37	210
391724.1	87	CIP38	211
392025.7	89	CIP45	212
392634.49	90	CIP49	213
392657.171	92	Demon	214
392740.4	93	Desiree	215
392745.7	94	Granola	216
392781.1	95	IWA 1	217
392821.1	96	IWA-2	218
392822.3	97	IWA-3	219
392973.48	98	Jelly	220
393079.24	99	NOHU 1301.01	221
393079.4	100	NOHU1301.03	222
393085.5	101	NOHU 1301.07	223
393248.55	102	NOHU 1301.08	224
393284.39	103	NOHU 1301.16	225
393371.164	104	NOHU 1301.20	226
393399.7	105	NOHU 1301.21	227
393613.2	106	NOHU 1301.25	228
393615.6	107	NOHU 1302.02	229
393617.1	108	NOHU 1302.18	230
393708.31	109	NOHU 1307.04	231
394034.65	110	NOHU 0702.18	232

APPENDIX- E (Continue) Name of genotypes used in thesis

Genotype Name	Genotype ID	Genotype Name	Genotype ID
394034.7	111	NOHU 0704.12	233
394223.9	112	NOHU 1204.03	234
394579.36	113	NOHU 1204.1	235
394600.52	114	NOHU 17	236
394613.32	115	NOHU 19	237
394881.8	117	NOHU 20	238
394895.7	118	Melody	239
395011.2	119	Russet Burbank	240
395017.227	120		

CURRICULUM VITAE

Muhammad Abu Bakar ZIA was born on September 10, 1986 in Muzaffargarh, Pakistan. He completed his higher secondary education from Government Degree College Muzaffargarh in 2005. Afterwards he joined Bahauddin Zakariya University, Multan Pakistan in 2005 for his undergraduate studies. He completed his B.Sc. (Hons.) in Agriculture from Department of Agronomy in 2009. After his B.Sc. (Hons.) he started his masters in the same department in 2009. In 2011 he graduated from the university and obtained GOLD MEDAL. He enrolled in Graduate School of Natural and Applied Sciences, Department of Agricultural Genetic Engineering at Niğde Ömer Halisdemir University, Niğde, Turkey to pursue his Ph.D. education under the supervision of Prof. Dr. Mehmet Emin ÇALIŞKAN. During his doctoral thesis research he worked on association mapping of some agronomic and morphological traits in potato.

